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THE MAMMALIAN SEX-RATIO

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CONTENTS.

I. INTRODUCTION.

THE factors governing the proportions of the sexes in mammals have been a subject of study since the earliest times, but, until a comparatively recent date, any real analytical consideration was made impossible by the absence of knowledge of the physiology of the reproductive processes, and particularly by the absence of any accurate data as to the manner of sex-determination. At the present time, though much is still obscure about reproduction and sex-determination, enough is known to warrant the statement that the factors governing the sex-ratio can at last be considered in a rational manner. The variations in the sex-ratio were originally used as the chief evidence relating to sex-determination, but, since the factors causing variations in the sex-ratio are very numerous, this way of approaching the problem led to the production of innumerable mutually incompatible theories,

and to hopeless chaos. Since one or other variation in the sex-ratio can be used to support any conceivable theory of sex-determination, it is clearly essential to consider the latter subject from a cytological and genetic standpoint, and to deal with the sex-ratio and its variations in the light of any conclusions arrived at. The aim, therefore, of this review is to present the subject of mammalian sex-ratio as it appears in the light of what is known of the cytology and genetics of sex-determination. The subject of sex-determination will therefore be considered first, and some of the older theories, since they are, for the most part, founded on interesting fluctuations of the sex-ratio, will of necessity be considered.

In the actual presentation of the material available one difficulty arises, and that is the diversity of forms in which the actual ratio is expressed. In the older work, the ratio was nearly always calculated as the number of males per 100 females. This system has the prohibitive disadvantage for new work that no reliable probable error can be calculated except for values near to 100. Two other systems are in vogue: the calculation of the males as a percentage of all cases, and the expression of the male ratio as a decimal of unity. Probable errors can be calculated for all values given by both these systems, and of the two the first is the less cumbersome, and probably the most convenient to use when presenting new matter. All three forms occur in the data to be discussed in this review, and comparison therefore is only possible by conversion, and in many cases this will be necessary.

II. THE ORIGIN OF THE MAMMALIAN SEX-RATIO.

In mammals the sexes are produced in almost equal numbers and such variations as are found are slight and do not alter the fact that the general nature of the mammalian sex-ratio is essentially one of equality. In the light of the respective reproductive capacities of the sexes this approximate numerical equality is extremely curious. It is impossible to doubt that from the point of view of the physiology of reproduction, one male is the potential equivalent of a considerable number of females, and this is increasingly true of species with a long period of gestation. The male produces many hundred millions of spermatozoa for every egg produced by the female and even though tens of millions of spermatozoa are used in effecting the fertilisation of one egg the male is left with a breeding capacity equal to many females.

In man the discrepancy between the reproductive capacities of the sexes is very obvious, and has been included by Metchnikoff among man's disharmonies, but the best demonstration is to be found in farm animals.

Stud bulls and stallions regularly produce upwards of 50 foals or calves in a season without harm to themselves or the progeny. The female, on the other hand, has a maximum capacity of one, or, rarely, of twins. Rams are given about 60 ewes to serve in two weeks, while the fertility of ewes, even under special agricultural methods, only averages three lambs in two years. In polytocous animals, i.e. animals having more than one offspring at a birth, the discrepancy between the reproductive capacities of the two sexes is less pronounced, but it is none the less real. In rodents, for instance, where the capacity of the female is huge compared with other groups of mammals, the capacity of the male is still infinitely greater.

From an evolutionary standpoint this discrepancy is of considerable interest. The potential productivity of a species could be enormously increased by the provision of a large excess of females, and since, as Herbert Spencer says, natural selection should tend to produce in a species the most advantageous sex-ratio, it is somewhat strange that the mammalian equality ratio still persists.

A reasonable explanation of the discrepancy would be provided if mammals were universally polygynous, in which case the struggle among the males consequent upon the endeavour to collect a retinue of females would result in the females going to the fittest males. In this way the difference in the reproductive capacities of the sexes would be turned to the advantage of the species. Polygyny, however, is anything but universal among mammals, so that this explanation is not sufficient.

If, then, the prevailing equality ratio bears no relation to the present needs of mammals and is not accounted for by competitive polygyny, it will be of interest to examine its possible origin. It may be said that the equality ratio is at the present time an hereditary feature of mammals and the inability of natural selection to change the ratio to a more productive type suggests that some deep-seated mechanism exists for maintaining the numerical equality. The chromosome mechanism, of course, fits in very well with this argument. Further, the general equality between the sexes in vertebrates suggests that the equality sex-ratio was among the characteristics inherited by mammals from the earlier vertebrates, who, showing little or no maternal care of young, required approximately as many males as females. It would appear, therefore, that the mammalian equality ratio, which has no relation to the present respective requirements of the sexes, may be regarded as an anomaly persisting, by virtue of its mechanistic basis, from the time before the evolution of maternal care in vertebrates when the female had a capacity equal to that of the male and when the sexes were, therefore, required in equal numbers.

III. THE DETERMINATION OF SEX.

A. *Old theories connected with the sex-ratio.*

(a) *Pre-natal conditions.* One of the oldest of all theories of sex-determination relates to the influence of the conditions of pre-natal life. Thus it was usually said that bad conditions during gestation led to an excess of males.

The forerunners of the essential parts of the reproductive organs of both sexes are present in all normal embryos, and this fact gives colour to the view that embryos are potentially hermaphrodite, or are neuter, as regards sex, and that environmental conditions, particularly those of nutrition, can accentuate one set of organs at the expense of the other set, or can sway neutrality to the male or female model.

It is obvious, however, that there are many grave objections to such an hypothesis. In the first place, considering the great variations in nutrition to which an embryo is subjected, one would have expected from this theory to find a great amount of variation in the sex-ratio. No such great variations are, however, usually found. As Pike [180] says, "Through periods of war and peace, of famine and of plenty, and under a great variety of racial and climatic conditions" the variation is usually very small. Other obvious criticisms are:

(1) One would expect that embryos developed together in a multiple gestation, under similar conditions, would be of the same sex. In polytocous animals this is clearly not true and the same applies to multiple births in what are normally monotocous animals.

(2) In animals which normally only have one at a birth, twins must each individually have less nutrition than a single embryo, and from this fact twins should tend to be mostly males. Again, there is no evidence that this is so.

(3) Light is thrown on the subject by a consideration of abnormal pregnancies. It occasionally occurs that extra-uterine pregnancies, such as ovarian, tubal, and abdominal gestations, are produced. Such pregnancies must of necessity be extremely badly nourished, and in some cases it has been possible to get the sex of the products of such gestations. Rauber[194] reports that nothing abnormal is found in the sex-ratio, and the fact that no excess of males is observable suggests that the malnutrition has no effect upon sex.

(4) The fact that the sex of an embryo is histologically observable at a very early stage of development, before the embryo makes any great call upon the mother for nourishment, seems to show that the time during which the environmental conditions of nutrition can have a chance of acting is very short.

(5) In the circumstances, however, the most damaging evidence against this theory is the evidence which tends to show that sex is determined not later than at fertilisation. Most important are the facts of polyembryony. In certain cases it is known that a fertilised ovum may split into two or more fragments and that each fragment may develop into a normal individual in the same foetal membranes. In these cases all individuals developing from a single ovum are found to be of the same sex, and the only explanation for this is that somehow or other sex is determined when the individual comes into being at fertilisation. In the human subject an appreciable proportion of twins are developed from a single ovum. These are known as identical twins and are of the same sex. In the case of the four-banded armadillo identical quadruplets are the normal thing, and here again the four individuals are always of the same sex. In certain Lepidoptera polyembryony runs to its extreme form, and in some cases several hundreds of individuals, always of the same sex, are developed from a single fertilised ovum.

(b) "*Vigour*" *theories.* Numerous theories, with and without support from sex-ratio statistics, have been put forward at one time or another postulating various sex-determining factors such as the relative vigour of the parents, the relative vigour of the gametes, the relative age of the gametes, etc. Such theories, however, are only of interest as regards the sex-ratio data brought forward in support of them. Their inherent improbability needs no comment.

(c) *The maturity of the ovum.* Considerable controversy has centred round the theory that the age of the ovum at fertilisation is the decisive factor in sex-determination.

The hypothesis that the length of time elapsing between ovulation and fertilisation is the sex-determining factor was elaborated by Thury in a series of papers [232, 233, 234].

On his view an ovum is first of all female as it is shed from the ovary, and then at some point in its passage of the genital tract it becomes male. Thus, if it is fertilised early a female is produced, and fertilisation later results in a male. During the change in the potentiality of the egg there must presumably be a neuter stage, and to fertilisation at this particular time Thury ascribes the production of abnormal hermaphrodites and intersexes. By working on this idea Thury claims to have been able practically to control the sex of farm animals. According to his experiments service during heat resulted in a great excess of heifer calves, and service at the end of heat in a preponderance of bulls. Baust (6) and Guiard (76) supported Thury, as also did Bell (11). Considering, however, that the exact relation between oestrus and ovulation is still clothed in obscurity, these experiments are founded on a doubtful basis. A peculiar case, mentioned by St Hilaire (209), has been invoked in support of Thury. A bitch was covered by two males of different breeds. The puppies which were similar to the first sire were all female, and those which took after the second sire were all male.

Pearl's work on the influence on the sex-ratio of the time of service during heat is instructive. In 1913, Pearl and Parshley (177) building on the earlier work of Russell (202) published the following statistics:

Table I. *Sex-ratio and time of service in cattle.*

Time of coitus	Total offspring	Males	Females	Sex-ratio
Early in heat	248	123	125	93·4
Middle of heat	125	67	58	115·5
Later in heat	107	65	42	154·8
Total	480	255	225	113·3

From these data the authors deduced that the time of service had an appreciable influence on the sex-ratio, although they did not subscribe to Thury's theory.

With a view to further testing this question Pearl proceeded with the collection of statistics, as a result of which the hypothesis was abandoned.

The following quotation is from the later paper (Pearl (173)):

Some earlier statistics appeared to indicate that there was a possibility of influencing the sex-ratio by paying attention to this point. It was believed to be of such extreme importance as to justify the careful study of the matter on the basis of much more extended statistics. These statistics we have now collected and analysed and shall publish as soon as possible. In the meantime it may be reported that, with the more extended statistics in hand, it appears to be conclusively established that there is no definite or permanent relation between the time in the heat period at which the cow is served and the sex of the offspring. The apparent relation between these two factors, which is believed by many breeders to exist and which our earlier statistics appeared to indicate, seems now to be purely accidental, and to have arisen only because of the comparative meagreness of the statistics on which the matter was discussed.

More recently this point has been investigated by Cooley and Slonaker [38] in the albino rat. By means of the vaginal smear technique[1] the exact stage of oestrus at which copulation took place was noted, and though interesting results were obtained as to the influence on fecundity, fertility, etc. the offspring from late and early breeding showed no appreciable difference in sex-ratio. The actual figures obtained were: early breeding, 114 males per 100 females; late breeding, 110·5. On the numbers of young obtained this difference is clearly not significant.

These investigations on mammals should be clearly distinguished from those made by Hertwig [92], Kuschakewitsch [117] and others on alteration of the sex-ratio in Amphibia by allowing the eggs to over-mature. In spite of the efforts to correlate the two sets of experiments, the lack of any real or conclusive evidence in the case of mammals invalidates any such attempt. In spite of this, however, Riddle [197] states:

The greater production of males in cattle from eggs that have remained un-fertilised for a period of hours is almost certainly correlated with an increased water content which the eggs obtain before fertilisation. We do not know by direct observation that the ovum of the cow takes up water from the fluid it meets in the reproductive passages. We do know that this is true for the eggs of every amphibian, reptile and bird that has been investigated.

In the case of the human subject the evidence is even more conflicting than that relating to the lower mammals, and particularly so because at the time when most of the data were collected little or nothing was known concerning the correlation between ovulation and menstruation, the time of conception always being con-sidered in relation to the latter. It now seems fairly certain, however, that ovulation occurs about the middle of the intermenstrual period. The supposed high masculinity of Jews has been explained on the ground that by the old Mosaic laws coitus is for-bidden for seven days after menstruation, and that the ova are, therefore, over-ripe, and potentially male. Pearl and Salaman [178], however, could find no evidence that in the human race the time of fertilisation of the egg relative to the catamenial period has any influence on the sex-ratio exhibited by the offspring. Siegel [216], in his first investigation on the relation of sex to the time of impregnation in the human female, found the following results:

Table II. *Maturity of human ovum (Siegel).*

Time of impregnation (days after menstruation)	Sex		Sex-ratio
	Male	Female	
1– 9	37	7	528
10–14	4	9	44·3
15–22	3	20	15
23–28	—	—	—
Total	44	36	122·1

[1] In certain mammals the period of oestrus is characterised by cornification of the vaginal epithelium and regular examination of the vaginal contents thus enables oestrus to be detected with great accuracy.

Fürst(68), also, says that impregnations during the four days after menstruation give an excess of males, and that impregnations during the days after that give a preponderance of females, as do immediately pre-menstrual impregnations. Pryll(190), however, failed to find any variation. Freeborn(67) found that conceptions during the first half of the intermenstrual period gave an excess of females: during the second half an excess of males. Bolaffio(26) obtained precisely opposite results.

In all such data, however, there appears to be a disturbing factor. Ewart(64) calls attention to the fact that male foetuses are some five or six days older at birth than female ones, and that this would appear to show that conceptions of males occur before those of females. In a later paper(217) Siegel himself comes to the conclusion that his first results were brought about by this factor, and says that in a later investigation he found that the average length of gestation of males was 272·6 days, as against 267·5 days for females.

(d) *Dimorphism of mammalian ova.* Many attempts have been made to show that the ova of mammals are dimorphic, one type producing females and the other type males, and such hypotheses have usually been involved with the assumption that one ovary produces one type only. Alternate ovulation of male-producing and female-producing ova has also been assumed in most of such cases.

Hippocrates (460-377 B.C.) considered that sex was determined in the ovary, that eggs from the right ovary produced males and that eggs from the left ovary developed into females. In 1895, Seligson(215) elaborated the hypothesis, and still later it was resurrected by Dawson(52). From a mass of clinical material apparently selected to support the theory, this last author asserted that ova from the right ovary produce males, and ova from the left females. His other assertions include: that the right ovary is larger and therefore certain to yield more ova than the smaller left; that the right Fallopian tube is larger than the left, and that its anatomical situation ensures for it a more facile receptivity; and that the *decubitus* in European races assists the fertilisation of ova from the right side. The accessory assertions explain very nicely the inequalities of the human sex-ratios if the left and right ova proposition be assumed, but the primary assumption is purely hypothetical, and can certainly not be true for other mammals. Copeman(39) has disproved it in mice, and Doncaster and Marshall(54) found that unilateral ovariotomy, which would presumably eliminate one type of ova, had no effect on the sex of litters in rats. King(105), also, has shown by unilateral ovariotomy that in the rat each ovary produces ova capable of developing into either sex. The only sort of experimental support for the theory are the very old and now discredited experiments of Henke(89). If the theory were correct for animals with bifid uteri it might be expected that male foetuses would be found in one cornu and females in the other. It has been shown by many authors, including Parker(150) and the present writer(163, 165), that this does not happen. However, as Doncaster and Marshall remark, disproof for various lower animals does not necessarily disprove a theory relating to man, but it detracts very seriously from its probability, and in any case some evidence relating to ovariotomy in man is to be found. In a number of cases of surgical semi-ovariotomy recovery has been

followed by the birth of children of the other sex to the one which would be expected from Dawson's view. Such a case was reported by Rawlings (195) where he removed the right ovary in a case of dysmenorrhoea, and subsequently a male child was born to the patient.

With these theories relating to dimorphism of mammalian ova must be mentioned Russo's work on rabbits. In a series of papers this author (203, 204, 205, 206) developed the idea that the eggs of rabbits are metabolically dimorphic, the anabolic ones producing females, and the katabolic ones males. In addition to this Russo claimed that one type can be transformed into the other by nutrition. He found normally in rabbits, and various other mammals, that the lecithin content of eggs of the same ovary varies greatly. This varying cell condition indicates a changing chemical state, expressed morphologically in the chromidial bodies. These structures, which he considers to be of great importance in development, Russo succeeded in producing artificially by injection of lecithin. Lecithin was administered:

(a) Subcutaneously,
(b) Interperitoneally,
(c) *Per os.*

The ovaries of treated rabbits became much larger.

His original evidence consisted of 100 selected litters from treated does, which gave 217 males and 431 females, a sex-ratio of 50·4. A hundred control litters from non-treated does were said to give 400 males and 287 females, a sex-ratio of 139·5. Criticism is obvious. As Castle (36) points out, the fact that the results were given on selected litters is enough to invalidate the deductions, and the sex-ratio of the controls, 139·5, shows that these were also selected. Heape (85) considers that Russo's male eggs were merely degenerate ones.

Working on Russo's methods Basile (8) and Punnett (192), giving all their results, came to negative conclusions. The former obtained 225 males and 215 females from controls, and 66 males and 51 females from treated does, females being in less proportion in the treated animals: Punnett got a sex-ratio of 110·0 from 153 rabbits, of which 24 males and 23 females were bred from treated does. Obviously, then, Russo's work is unconfirmed, and can only be regarded as unproved.

B. *The chromosome mechanism.*

The chromosome theory of sex-determination is so widely known and so generally accepted at the present time that no detailed description need be given here. Considering the case of mammals it may be said at once that the evidence for the chromosome theory in this group is neither as profuse nor as authentic as in lower animals (particularly insects), but there seems little reason to doubt that the mammalian male is heterozygous for sex and produces two types of spermatozoa, while the female is homozygous and produces ova which are all of the same chromosome constitution. Evidence from the inheritance of sex-linked characters is unfortunately scarce in mammals, and at the same time the large

numbers of chromosomes make cytological work difficult, but in spite of this an impressive list of mammals in which the male is known to be heterozygous can be put together. In addition, further confirmatory evidence has been obtained by showing that dimorphism of the spermatozoa head-lengths exists in several cases. The following table, taken from Huxley (100), summarises well the present state of the evidence for supposing that mammals produce spermatozoa of two types— one type being male producing and the other female producing:

Table III. *Male heterogamety in mammals.*

Species	Chromosomal evidence	Sperm-head bimodality	Genetic evidence
Man	XY Painter	+ Parkes (154)	+
Monkeys (spp.)	XY Painter	...	–
Bull	XO Wodsedalek	+ Wodsedalek (247)	–
Horse	XO Wodsedalek	+ Wodsedalek (246)	–
Pig	XO Wodsedalek	...	–
Dog	XO Malone	+ Zeleny and Faust (250)	–
Cat	XO v. Winiwarter and Sainmont	– Parkes (154)	+
Rabbit	XY Bachuber	+ Huxley and Baker	–
Guinea-pig	XY Stevens	...	–
Rat	XO Allen	+ Parkes (154)	–
Mouse	XO Yocum	+ Parkes (154)	–
Opossum	XY Painter	...	–

C. *Sex-differentiation.*

In the normal way there can be little doubt that the sex-differentiation of the zygote follows the chromosome constitution, but the means whereby a mammalian zygote of XX constitution is moulded to a female, and one of XY constitution to a male, is as yet not certainly known. The volume of experimental work which has been performed on the lower animals with a view to throwing light on this problem has failed to produce any coherent explanation. In mammals the final differentiation of sex is dependent on hormonic stimuli from the gonads, but as these stimuli again are dependent on the presence of normal gonads the whole course of sex-differentiation, even in mammals, is essentially traceable to the original constitution of the zygote. Cases are, however, known where the chromosome constitution is over-ridden and sex-reversals or intersexes are produced. These belong to two main types, (a) zygote intersexes where the original differentiation is disturbed, and (b) hormonic intersexes or pseudo-intersexes where the hormonic action of the gonad is disturbed by outside influence (*e.g.* the free-martin) or is delayed. Such cases are not, however, sufficiently common in mammals (it is not known if (a) occur at all in mammals) to complicate the assessment of the sex-ratio, and from the standpoint of this review the chief interest in abnormalities of sex-differentiation lies in possible effects on the sex-ratio of breeding from sex-reversals, that is to say, in breeding from individuals functioning as one sex with the chromosome constitution characteristic of the other. The elaboration of the accessory organs of reproduction in the mammal make it highly improbable that

adults could be reversed to functional specimens of the other sex, but if the process of reversal began early in embryonic life, before the accessory organs were irrevocably set upon one path or the other, complete reversal might be achieved. Whether this ever occurs naturally is not known (most apparent mammalian intersexes appear to be cases of delayed hormone action), and its experimental production seems to be beyond a practical proposition—as yet. The influence of functional sex-reversals on the sex-ratio will be discussed later.

IV. THE SEX-RATIO THROUGH THE LIFE CYCLE.

A. *Implications of the chromosome theory.*

The primary implication of the chromosome theory of sex-determination is that sex is determined at the time of fertilisation, and it is therefore justifiable to speak of a sex-ratio from this time onwards, right through gestation to the oldest ages to which individuals live. Two other implications of importance are:

(*a*) Since the two types of spermatozoa are produced in equal numbers, it would be expected that the two sexes would be conceived in equal numbers.

(*b*) The only changes which can come about in sex-ratio must be brought about by mortality (except in the rare event of abnormality of sex-differentiation).

In the light of these three implications of the chromosome theory the whole question of the sex-ratio may be attacked.

B. *The ratio at birth.*

Since a sex-ratio exists right from conception onwards, and since there is no reason to suppose that the sex-ratio should remain the same through the life cycle, an accurate study of the variation during the cycle would be of great interest, but owing to the paucity of data (even in the human subject) the best that can be attempted at present is to consider the ratio at the three salient points: conception, birth and maturity. The ratios at these three points are sometimes known as the primary, secondary and tertiary ratios respectively. Even when the study of fluctuation during the life cycle is reduced to this mere outline, immediate difficulties arise. In the case of mammals which can be experimented upon, statistical data on a large scale are scarce, and the human subject, for whom statistical data are plentiful, is not available for experiment. In any case the ratio at conception cannot as yet be directly estimated, and it is necessary to consider the ratio at birth, find out as much as possible about the amount and sex-incidence of pre-natal mortality, and calculate backwards. As regards the ratio of adults, reliable data exist only for the human being, and these are complicated by emigration and immigration. Of the ratio at birth, however, considerable information is available and by calculating backwards and forwards with the knowledge that the ratio at birth is always very near to equality a lot can be done to elucidate the changes taking place in the sex-ratio during the life cycle.

C. *Post-natal changes in the ratio.*

The changes taking place in the sex-ratio after birth may first be considered. For this purpose it is necessary to know something relating to the numbers of each

sex eliminated during this time, and for this it is necessary to know (1) the amount, and (2) the sex-incidence of post-natal mortality. Human statistics are practically the sole source of material, and these two factors will be dealt with in order.

For the first two or three weeks of life the mortality is very heavy, and particularly so on the first day of life, as the analyses of both Düsing (58) and Pearl (172) show. During the whole of the first year the mortality is gradually declining (Düsing (58), Pearl (172), Bentzen (13)), but the details need not be entered into here, as the first year is the smallest unit that need be considered. For 1913 (the last pre-war year) the percentage mortality in different age groups was as follows (Registrar-General):

Table IV. *Amount of post-natal mortality in England and Wales*, 1913.

Age	Deaths per 1,000,000	Percentage
0– 5	32,472	3·24
5–10	3,057	·30
10–15	1,895	·18
15–20	2,752	·27
20–25	3,290	·32
25–30	2,863	·28
30–35	4,781	·47
35–40	6,370	·63
40–45	8,132	·81
45–50	11,084	1·10
50–55	15,157	1·51
55–60	21,835	2·1
60-65	31,935	3·1
65–70	30,579	3·0
70–75	76,207	7·6
75–80	112,159	11·2
80–85	327,912	32·7
85 +	511,631	51·1

This shows that the least deaths occur between 10 and 15 years of age and that the amount increases steadily from then onwards. It is interesting that the mortality during the first five years is very heavy, much the same as at 60–65 years of age, and is quite enough to affect the sex-ratio if unevenly distributed.

The sex-incidence of post-natal mortality in man is well known, the popular verdict being that boys are harder to rear than girls. Prinzing (188) gives the following table of the sex-ratio of mortality in the first year of life:

Table V. *Sex-ratio of mortality in first year of life.*

Country	Sex-ratio	Country	Sex-ratio
Italy	111	England	121
Rumania	115	Sweden	121
France	119	Denmark	121
Austria	119	Norway	123
Switzerland	120		

Kroon [115] states that in Holland the sex-ratio of mortality in the first year of life is 119, and that for the first two months of life it may even be as much as 139.

The sex-ratio of mortality for the first year of life in England and Wales (1913) was as follows:

Table VI. *Mortality during first year of life, by age and sex*, 1913.

| Age | Deaths per 1000 | | Sex-ratio |
	Males	Females	
0– 3 months	67·47	51·79	131·5
3– 6 ,,	21·64	17·82	121
6– 9 ,,	16·81	14·04	120
9–12 ,,	14·22	12·59	113
Total year	120·14	96·24	125

These results are confirmed by the work of Düsing [58], Rauber [194], Prinzing [188], Nichols [147], Dutton [60], Pinard and Magnan [181], Kroon [115], Ashby [4] and Davis [49, 50, 51], who all found that more boys than girls die under one year old, but that the excess decreases through the year.

Most authors agree that for the first five years of life the sex-ratio of mortality is above equality, that more males die than females. It seems equally certain that soon afterwards the girls begin to die faster than the boys. Thus Kroon [115] says that between 14 and 15 years of age as few as 80 males die per 100 females, and Prinzing says that the sex-ratio of mortality between 5 and 20 years of age is below equality. The records of England and Wales, 1913, show that between 5 and 10 years of age the ratio is only just equality (100·7) and that for 10 to 15 years old it is as low as 93·3.

Schultz [212] puts this down as chiefly the result of tuberculosis, "for which disease the common occurrence of anaemic and chlorotic conditions at the time of puberty furnishes an excellent soil. After this age the sex-ratio of mortality increases rapidly and results in the reversion of an excess of females to an excess of males." The general low mortality at this period (see Table IV) does not, however, seem to support this last contention, which is also deprecated by Knöpfel's [111] assertion that between 25 and 40 years of age the sex-ratio of mortality is below equality in rural districts and above in the urban populations.

Stewart [223], however, found that between the ages of 10 and 20 years the deaths from pulmonary tuberculosis show a huge excess of females, while at other times males preponderate. This seems to support Schultz's suggestion.

In any case it is clear that the excess of female deaths at this time is due to the fact that the onset of puberty is more severe in the female.

The full data for England and Wales, 1913, are appended below:

Table VII. *Post-natal mortality in England and Wales*, 1913, *by age and sex*.

Age	Deaths per 1,000,000		Sex-ratio
	Males	Females	
0– 5	35,495	29,449	113·4
5–10	3,068	3,047	100·7
10–15	1,823	1,968	93·3
15–20	2,816	2,688	104·9
20–25	3,523	3,058	115·3
25–30	4,174	3,552	117·5
30–35	5,207	4,356	119·6
35–40	6,957	5,783	120·1
40–45	9,117	7,146	127·5
45–50	12,522	9,646	132·5
50–55	17,250	13,064	132·0
55–60	24,784	18,886	131·1
60–65	36,405	27,465	132·8
65–70	51,140	40,018	128·0
70–75	83,568	68,846	121·0
75–80	121,487	102,830	121·0
80–85	175,703	152,209	115·0
85 +	266,022	245,609	113·0

Some values have now been given for both of the two factors which govern the adult ratio, *e.g.* the amount and sex-incidence of post-natal mortality, and it is possible to consider the adult ratio in the light of these data.

D. *The ratio among adults.*

The gross ratios for countries and continents have very little biological meaning, but before dealing fully with England and Wales in the light of the factors discussed above, it may be of interest to consider a few figures for the world.

In 1911 the population of the world was 1600 millions. 1000 millions of this total was from census reports, and accurate data for this number are therefore available. The figures for Europe, which were complete with the exception of Turkey, showed an excess of 8 millions of females in a population of 480 millions, a sex-ratio of 96·8. America, with a total of 170 millions, gave an excess of 4 millions of males, or a sex-ratio of 104·8.

In the 400 millions of Asia, for which figures are available, there was a male excess of 9 millions, the sex-ratio in this case being 105·2. Of Africa's 127 millions, the sex-distribution of only 17 millions was known, there being an excess of females among the negroes, and of males among the whites. In India, an excess of males amounting to 9 millions was found in the population of 300 millions, a sex-ratio of 106·9.

Table VIII, quoted from Bälz[7] with the ratios recalculated as males per 100 females, was compiled from the census of 1910.

In 1911 the only European countries which showed a gross excess of males were the Balkan States and Luxemburg. Every other country showed an excess of females, which was actually greatest in Great Britain and Russia, and relatively

Table VIII. *Sex-ratio of entire population.*

Country	Males per 100 females	Country	Males per 100 females
Great Britain	93·5	Belgium	98·4
Norway	94·0	Italy	99·0
Denmark	94·5	Poland	100·5
Sweden	95·3	Greenland	101·5
Spain	95·3	Japan	102·0
Austria	96·6	India	104·1
Germany	96·9	Bulgaria	104·5
European Russia	97·2	Serbia	106·0
Switzerland	97·2	Siberia	106·0
Hungary	97·7	Caucasus	111·0
France	97·9	Korea	113·0
Holland	98·2	Asiatic Russia	117·5
Ireland	98·3	China	125·0

the greatest in Portugal and Norway. In Ireland, Germany, Finland, Sweden and Switzerland the excess of females was less in 1911 than in previous census years, but in England, Belgium, Denmark, France, Italy, Norway, Portugal and Spain a persistent rise in the excess was observable.

The chief biological interest, however, in entire populations lies in the sex-ratios of the various age groups, and the following table gives the relevant data for 1913:

Table IX. *Estimated population of England and Wales, 1913, by age and sex.*

Age	Males	Females	Sex-ratio males per 100 females
0– 1	404,705	396,124	102 0 ⎫
1– 2	383,236	377,804	101·5 ⎬ 101·0
2– 5	1,195,270	1,191,534	100·3 ⎭
5–10	1,891,801	1,894,602	99·9
10–15	1,788,908	1,894,602	94·2
15–20	1,693,711	1,721,461	98·5
20–25	1,538,431	1,712,214	89·7
25–35	2,899,804	3,198,584	81·4
35–45	2,391,988	2,568,336	89·7
45–55	1,732,989	1,875,472	92·2
55–65	1,109,286	1,240,145	89·3
65–75	615,852	774,011	79·4
75–85	187,815	275,815	68·0
85 +	23,218	42,158	55·2

Thus the ratio, which at birth was 104, is reduced at the end of the first year to 102, while at the end of the second year of life further reduction has taken place to 101·5. In the third to fifth years this has decreased to 100·3, the average for the first five years of life being 101. From 5 to 10 years of age, the ratio is further decreased to 99·9, while during the following five years a further decrease to 94·2 takes place. For 15 to 20 years of age, however, a rise is seen, following upon the increased mortality of females during the previous five years. From this point the proportion of males shows a continuous decline, with one small exception, right

on to old age; the exception is a slight rise between 40 and 50 years of age following the slightly increased mortality of females at 40 to 45 years of age. During old age the excess of females is really striking, and at the age of 85 and over the sex-ratio of about 65,000 individuals is only 55·2 males per 100 females.

E. *Pre-natal changes in the ratio.*

To be able to make an estimate of the proportions of the sexes at conception it is necessary to know something about the amount and sex-incidence of pre-natal mortality. The method of computing the amount of pre-natal mortality depends on the means whereby a dead conceptus is removed. In polytocous animals where the abortion of one dead foetus would mean the sacrifice of the whole litter, the death of a single foetus does not seem to set up the stimuli necessary for abortion; the result is that the dead foetus starts to autolyse and may be entirely reabsorbed by the mother with the exception of the bony and horny parts. This process has been found to occur in pig, rabbit and sheep (Hammond (79, 80)); ferret, marmot and mole (Müller (144), Strahl and Henneberg (226)); pig (Meyer (141, 142), Corner (41)); dog and cat (Kuntz (116)); ferret (Robinson (199)); rat (Huber (97), Long and and Evans (127)); mouse (Parkes (157), Long and Parkes (128)) and other polytocous mammals. In monotocous mammals, however, the death of a foetus, especially if well advanced, seems normally to result in abortion.

The number of foetuses which die and are reabsorbed is difficult to estimate, but rough estimations may be made by two methods: (*a*) comparison of number of normal foetuses, atrophic foetuses and corpora lutea found on autopsy; (*b*) comparison of size of litter at birth and number of corpora lutea. Summary tables

Table X. *Difference between number of foetuses and number of corpora lutea.*
(Various authors.)

Author	Animal	Foetuses found		Corpora lutea	Percentage death and reabsorption
		Normal	Atrophic		
Hammond (79)	Sheep	101	8	116	12·9
Hammond (80)	Pig	267	49	396	32·5
Robinson (199)	Ferret	1246	—	1643	24·2
Corner (41)	Pig	3442	43	4480	23·3
Parkes (157)	Mouse	76	—	82	7·3

Table XI. *Number of corpora lutea and size of litter at birth.*
(Various authors.)

Author	Animal	Average size of litter at birth	Average corpora lutea	Percentage mortality
Long and Evans (127)	Rat	6·7	10·0	33·0
Hammond (80)	Pig	12·0	20·0	40·0
Robinson (199)	Rabbit	5·0	9·6	37·5
Biedl, etc. (21)	Rabbit	6·9	9·11	18·33
Robinson (199)	Ferret	6·0	9·95	39·23

(quoted from a previous paper) showing the results obtained by these methods are given above.

The results given by the first method do not necessarily include all pre-natal mortality, while the second method includes even still-births in most cases. The higher percentage mortality shown by the second table is therefore accounted for. Both methods, of course, give too high results in so far as unfertilised ova are included, but even so it is obvious that pre-natal reabsorption of foetuses is normally considerable, and may in cases involve a large proportion of conceptions.

Where dead foetuses are aborted, some index of the amount of pre-natal mortality may be obtained by comparing the number of abortions observed with the number of normal births. Data of any value have only been collected for the human subject by this method, and even in this case it is clear that nothing like the full amount of pre-natal mortality can be assessed, especially since embryonic death during the early stages will result in no obvious abortion, and will in any case remain for the most part unrecorded. However, using the incomplete data obtainable, various investigations, summarised in the following table, have been made.

Table XII. *Amount of abortion.*

Author	Animal	Amount of abortion (percentage of all pregnancies)
Routh (201)	Man	20·0
Priestley (186)	,,	23·0
Whitehead (242)	,,	14·0
Williams (244)	,,	16 2
Franz (66)	,,	15·4
Malins (136)	,,	19·2
Taussig (228)	,,	30·3
Pearson (179)	,,	28·5
Rauber (194)	,,	9·6
Auerbach (5)	,,	24·0
Parkes (158)	,,	16·5
Davies (48)	,,	15·6
Bertillon (17, 18)	,,	6·8
Mall (137)	,,	20·0
Ahlfield (2)	,,	20·0
Schultz (212)	,,	22·0
Goehlert (72)	Horse	6·0*
Heape (84)	Cow	10·0*
Heape (83)	Sheep	2·3*

* The data for farm animals must be very incomplete.

The extent to which pre-natal mortality may increase under certain conditions is shown by Bluhm's[23] statement that in Germany, in 1915–16, 190 abortions occurred to every 100 full-time births among working women, a state of affairs attributed by Bluhm to the conditions of labour.

Certain data are available relating to the distribution of abortion in the various stages of gestation.

Williams considers that the percentage would be much increased if it were possible to allow for the early abortions which show only as retarded and profuse

menstruation, and Auerbach assumes that many of the early ones are unnoticed. Even so he found that of the recorded ones half fell within the first three months of pregnancy. The early incidence of the majority of abortions is confirmed by Rauber (194), Nichols (147) and Carvallo (35). Franz (66) says that in the third month of pregnancy alone 42·6 per cent. of the total abortions take place, while Dühressen (55) puts the percentage for this month at the high figure of 57. Günther (77) considers that both amount and sex-ratio increase logarithmically back into gestation.

To conclude the data relating to the amount of pre-natal mortality it is necessary to consider the frequency of still-birth. This is shown in Table XVII, together with the sex-incidence.

Taking the above data as a whole it seems possible to conclude that in mammals one out of every four or five conceptions is destined not to reach full term and if the sex-ratio of those eliminated before birth is much different from the ratio at birth, it means that considerable change may take place in the sex-ratio during gestation.

The assessment of the sex-incidence of foetal reabsorption is a matter of some difficulty. Even in an animal such as the pig where the sex of foetuses is distinguishable at a very early age, it is usually impossible to ascertain the sex of partially reabsorbed ones, and in mice and rats it is even less possible to find the sex-incidence directly. However, if it is assumed that the sex-ratio at conception is fairly constant, the sex-ratio at birth would vary according to the amount of mortality if the mortality falls unequally upon the two sexes. An investigation by the present writer on a small amount of material suggested such a correlation. The actual figures were:

Table XIII. *Pre-natal mortality and sex-ratio at birth in mouse* (Parkes (157)).

Mice	Loss of ova per 100 foetuses	Sex-ratio males per 100 females (at birth)
Normal	10·8	118·0
Gestation while lactating (short period)	17·6	80·4
Gestation while lactating (long period)	23·1	62·1

These figures suggested immediately that the pre-natal elimination fell preponderatingly on the males, but much more extensive work of the same type by MacDowell and Lord (130) failed to show the same correlation. Their figures are as follows:

Table XIV. *Amount of pre-natal mortality and sex-ratio at birth in mice* (MacDowell and Lord (130)).

Percentage pre-natal mortality	0–19·9	20–39·9	40–59·9	60–79·9	80–99·9
Males per 100 females	102·9	100·0	102·7	103·4	63·6

As regards the sex-ratio of human abortions more is known. The following table is computed from various authors:

Table XV. *Sex-ratio of abortions.*

Authority	Age of abortion	Number	Sex-ratio
Lenhossek (120)	3–6 months	156	160·0
Auerbach (5)	4–7 months	4067	156·4
Carvallo (35)	Up to 4th month	—	250·0
Günther (78)	—	—	143·2
Kŏrösy (114)	—	3781	152·4
Pinard and Magnan (181)	—	1229	101·1
Rust (207)	First 6 months	454	101·8
Rauber (194)	—	—	159·0
Schultz (212)	3–10 months	647	118·7
Marmisse (138)	—	2881	144·7
Bertillon (18)	—	—	121·5

The males are thus apparently definitely preponderant among abortions. A number of investigations have been made of the variation in the sex-ratio of mortality at different stages of gestation. Table XVI summarises these:

Table XVI. *Sex-ratio of mortality according to month of gestation.*

Month of gestation	Sex-ratio according to					
	Auerbach (5)	Schultz (212)	Schultz (213)	Nichols (147)*	Marmisse (138)*	Bertillon (18)*
1	—	—	—	—	—	—
2	(452)†	—	—	—	—	—
3	(322)†	123·7	121·0	} 176	170·0	180·3
4	229	110·5	117·5	}		
5	163	108·1	109·6	148	105·0	118·6
6	116	58·8	87·5	111	119·5	112·0
7	116	—	108·5	114	113·2	116·5
8	—	—	133·3	106	136·6	106·7
9	—	—	167·6	129	132·5	131·6

* Calculated from their data.   † Interpolated figures.

In many cases these results are, however, arrived at on comparatively small numbers and none of these authors appear to have considered the statistical significance of their figures. From the point of view of numbers, Auerbach's work is far the most convincing. Much material is also presented by Tschuprov (236).

Finally, it remains to consider the question of still-births. Table XVII summarises data relating to this point:

Table XVII. *Frequency and sex-incidence of still-births.*

Authority	Locality and date	Percentage	Sex-ratio
Bodio (25)	1887–95	—	
	Italy	—	131·1
	France	—	142·2
	Germany	—	128·3
	Austria	—	132·1
	Hungary	—	130·0
	Switzerland	—	135·0
	Belgium	—	132·0
	Holland	—	127·1
	Sweden	—	135·0
	Norway	—	124·6
	Denmark	—	132·0
Dawson (52)	—	—	138·0
King (108)	America	4·36	131·06
Nichols (147)	All records	3·04	131·6
Lewis (121)	Europe	—	120–170
Heape (87)	Cuba	—	144·45
Hirsch (93)	Germany	—	127·9
Davis (49, 50, 51)	United States	5·56	137·1
Dusing (58)	Prussia	4·06	129·09
Quételet (193)	—	—	133·5
Terry (229)	Massachusetts	3·2	—
Rauber (194)	Germany	4·0	—
Auerbach (5)	Budapest	3·3	—
Le Maire (134)	Copenhagen	5·7	—
Bucura (30)	Vienna	5·8	—
Bertillon (17)	—	4·5	—

For the sex-ratio of still-births in lower mammals data are very scarce. King (108) gives the following totals for the sex-ratio and percentage of still-births in the rat:

Table XVIII. *Sex-ratio of still-born rats* (King (108)).

Number of litters with still-births	Number of young	Males	Females	Sex-ratio	Percentage of total young in litters
253	1817	234	181	129·3	22·8

The sex-ratio of still-births in rats is, therefore, remarkably similar to that of man.

Goehlert (72) gives 106–107 as the sex-ratio of still-births in the horse, as against the birth sex-ratio of 96·57.

There is thus good ground for Günther's generalisation that both the amount and male excess of pre-natal mortality increase going back towards conception. Since all authors agree that, in man at any rate, pre-natal and immediately post-natal mortality falls more heavily on the males, it is necessary to consider why this should be so.

Carvallo (35) says, "les garçons sont les plus fragiles," a conclusion supported by Auerbach (5). Grassl (74) considers that the difference is connected with the germ-plasm. Rauber (194) explains the greater mortality of the males on the grounds

of their greater size making greater demands on the mother, and the consequent greater possibility of the demands being unsatisfied. As, however, most abortions occur early on when the embryo makes least demand on the mother, it is difficult to believe that nutrition has much power of regulating the amount and nature of abortion. Darwin[45] and later Havelock Ellis[62] noted in this connection that males are more variable than females, and are therefore likely to present more lethal abnormalities. Ewart[63] suggests that the female conception may graft itself more easily upon the uterus than the male. Theories built on the supposedly larger size of the male foetus are, however, the most common. Prinzing[189] found that operative measures at birth are taken in 6·18 per cent. of male births and in only 4·67 per cent. of female. This, however, is not necessarily due to size of child, as Von Winckel[245] found that in excessively heavy infants operative measures were not taken in any greater excess of cases than in lighter ones, and that in these very heavy ones death was little above the normal.

It seems difficult, also, to see how the slight size difference which exists between male and female new-born in animals like the rat and mouse[1] can have any decided influence on the mortality. The fatal point about any size hypothesis, however, is that the excess male mortality keeps up after birth when a slight difference in size can have no influence. Huxley has been led to suggest that some sex-linked semi-lethal factor is concerned.

As regards the high male proportion among still-births size hypotheses are more reasonable. It is often said that the characteristic sex-ratio of still-births is due to the general larger size, and particularly to the larger head size, of male foetuses, and Dutton[60] adds that cranial ossification is more advanced in the male than in the female. This author also says that the pelvic development of women is not proportionate to the cephalic development of foetuses, and Bluhm[22] supports this by the fact that medically induced prematurities are on the increase. If this be so a more compressible head due to less ossification would be an advantage to the female. The supposition that the masculinity of still-births is due to size is also corroborated by Hoffmann[96], Dohrn[53] and Orschansky[149] who found that the sex-ratio of offspring of women with narrow pelvises was low. There are, however, well-authenticated facts which contradict this argument. Firstly, labour troubles are more common with primiparae than with multiparae, but still-births are more common with multiparae (Parkes[158]), so that still-births are presumably mostly dead before parturition sets in and are not mostly due to actual labour troubles. This theoretical supposition is confirmed by Ladame[118] who found that only 36·4 per cent. of still-births died during birth. Size, too, can play but little part in prematurities, and Treichler[235] and Prinzing[189] found respectively 29·6 per cent. and 32·6 per cent. of still-births to be premature. Finally, Linden[124] found that in 360 births from mothers with contracted pelvises the sex-ratio was 133, a finding which contradicts the authors who found such ratios to be low.

In conclusion it can only be said that the factors causing foetal elimination to differentiate between the sexes are as yet obscure.

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[1] Rat. Jackson[101] says males 5·7 gm. at birth, females 5·4.

F. *The ratio at conception.*

From the fact that an appreciable amount of pre-natal mortality takes place, and from the further fact that this eliminates considerably more males than females, it is to be presumed that the sex-ratio which is about equality at birth shows an increasing preponderance of males back towards conception. At conception the male proportion should be higher than at any other time in the life cycle. It is, of course, impracticable to test the ratio at conception directly, but some information may be obtained by finding the sex-ratio of foetuses at different stages, going as far back towards conception as possible. Owing to the difficulty of distinguishing the sexes of foetal rodents most laboratory mammals are useless material for this kind of work, but some data relating to farm animals exist.

Lillie (122) in his work on the free-martin found that the foetal sex-ratio for dizygotic twinning in cattle was 134, as compared with a birth ratio of 100–110 (see later). Jewell (104) found that the sex-ratio of 1000 cow foetuses was 123·21, but he was unable to correlate difference in the sex-ratio with different stages of development.

The present writer (Parkes (165)) found a male percentage of 56·8 ± 1·38 among 583 pig foetuses of all ages. This percentage was much higher than that of 49·56 ± ·065 for combined figures for pigs at birth given by various authors. In addition three size groups showed an inverse correlation between male percentage and stage of development. The actual figures were:

Table XIX. *Weight of foetus and sex-ratio in pigs.*

Weight group	Total	Males	Females	Percentage males
0–100 gm.	281	166	115	59·1 ± 1·98
101 ·300	114	65	49	57·0 ± 3·12
300 +	188	100	88	53·2 ± 2·45
Total	583	331	252	56·8 ± 1·38

From these figures it would seem that the ratio at conception in the pig must be very near 60 per cent. of males, or 160 males for 100 females. These results have been confirmed by Crew (42), who also investigated pig foetuses, and came to similar conclusions.

From the data obtained as to (*a*) sex-ratio at birth, (*b*) amount of foetal mortality, (*c*) sex-incidence of foetal mortality, various attempts have been made to calculate a probable figure for the ratio at conception in man. Some of these computations are given below:

Table XX. *Sex-ratio at conception in man.*

Author	Sex-ratio	Author	Sex-ratio
Bernouli (15)	108·2	Auerbach (5)	116·4
Jendrassik (103)	108·7	Dawson (52)	110·0
Lenhossek (120)	111·0	Schultz (212)	108·47

Of these, however, Auerbach maintains that the figure would be much higher (125) if the early abortions could be estimated.

The only evidence that the ratio at conception is not much higher than that at birth is an investigation by MacDowell and Lord (129) on the sex-ratio of litters which were found by corpora lutea counts to have suffered no pre-natal mortality. The number of such litters obtained was 68, giving 523 young. Of these young 261 were male and 262 female, giving 99·8 males for 100 females, a ratio indistinguishable from the normal ratio at birth. At the same time there is no proof that these litters represented a random sample of conceptions. If males were less viable than females, litters with the most females would tend to escape pre-natal mortality to a greater extent than those with an excess of males, and hence females with an original surplus of females would tend to be represented preponderantly among these data. So far as the human subject is concerned there seems to be no doubt that the ratio at conception has a greater proportion of males than at birth, and since a small excess of males is actually found at birth, a fairly appreciable excess of males (see Table XX) must exist at conception.

There is thus some discrepancy between the chromosome theory of sex-determination with its corollary that the sexes should be conceived in equal numbers, and the fact that the sexes are not conceived in equal numbers. As King (108) says: "The fact that in man the sexes are very evidently not conceived in equal numbers is a decided stumbling-block in the way of any theory that postulates chance as the chief factor in deciding whether a given ovum shall become male or female." Nevertheless, the difficulty is far from insuperable. The only essential implication of the chromosome theory is that the X and Y spermatozoa are produced in equal numbers, and this does not necessarily mean that fertilisation takes place in the same ratio. Three distinct factors may come into action to upset the anticipated equality ratio at conception:

(a) The two types of spermatozoa may be differentially susceptible to adverse conditions and may therefore not survive the struggle for existence in equal numbers.

(b) The two types of spermatozoa may have a differential activity, resulting, possibly, from the head length dimorphism.

(c) Even assuming that the place of fertilisation is reached by equal numbers of the two types, the actual penetration of the ovum may be easier for one type than for the other.

That (a) and (b) may occur in practice is shown by a variety of facts. Dealing firstly with differential mobility of spermatozoa, there are Marshall's old experiments on artificial insemination with mixed semen. Pure-bred bitches were inseminated with semen of similar pure-bred dogs and semen of dogs of other breeds mixed together. The bitches subsequently produced mongrel pups, showing that the "alien" spermatozoa had triumphed in the race to effect fertilisation.

In this connection it is relevant also to refer to the peculiar custom of the mule breeders in Kansas mentioned by Robertson (198), of always putting a horse to a mare a few minutes before she is served by the ass stallion. This practice was

brought to light by the anomalous appearance of a mule and a horse as twins, but it seems that in the usual way mules only are produced and this argues that the spermatozoa of the ass have some advantage over those of the horse stallion. Other interesting facts bearing on the point can be found. Cole (37) mated the same doe rabbit at the same oestrous period with two bucks of different character. One male showed itself more potent by always having more young like it. When this stronger male was alcoholised to damage the sperm no young like it were produced, though it was found to be fertile when used alone.

These experiments show that the relative vigour of different spermatozoa may be altered by adverse conditions, and Heape's (87) observation that artificial insemination, which can hardly provide optimum conditions for the spermatozoa, tends to increase the proportion of males suggests that the male-producing spermatozoa withstand bad conditions more readily than the female-producing ones.

The experiments of Stockard and Papanicolaou (225) on alcoholic degeneration are also instructive in the question of differential vitality. It was found that the progeny of alcoholised males showed greater abnormality than those of alcoholised females, and that in the former case the daughters showed more ill-effects than the sons, presumably owing to the greater susceptibility of the female-producing spermatozoa.

It seems possible to conclude, therefore, that the excess of males at conception is probably brought about by virtue of the male-producing spermatozoa being more efficient fertilising agents than the female-producing spermatozoa, the actual difference being probably in activity and vitality.

V. VARIATION IN THE SEX-RATIO AT BIRTH.

A. *General considerations.*

It has been mentioned above that the only ratio for which a considerable amount of reliable data can be obtained is the ratio at birth, and it is of interest, therefore, to endeavour to analyse the causes of the variations which are found from time to time, from place to place, and from one set of conditions to another, in the proportions of the sexes at birth. This can only be done by considering the two factors which govern the proportions of the sexes at birth and by endeavouring to locate which one, if not both, has contributed the particular variation under discussion.

From what has been said above, it is clear that two factors govern the sex-ratio at any given time. These are

(a) The initial sex-ratio at conception.

(b) The amount of mortality and the sex-incidence of mortality between conception and the given time.

Hence the factors which govern the sex-ratio at birth are the sex-ratio at conception and the amount and the sex-ratio of the pre-natal mortality, and as has been pointed out before:

This means that variation in the sex-ratio at birth may be caused by a variation in either or both of these two factors. Thus, a heavy foetal mortality produces,

other things being equal, a low proportion of males at birth, while a low mortality during gestation decreases the excess male wastage, and raises the proportion of males at birth. On the other hand, where the amount of intra-uterine death is more or less constant, the sex-ratio at birth will be determined by that at conception. If both of these two factors vary together, however, the variation may be cumulative or compensating. Thus a high ratio at conception coupled with a low mortality during gestation will raise considerably the ratio at birth, whereas coupled with a high mortality it will produce a more or less normal ratio at birth. Conversely a low ratio at conception in conjunction with low foetal mortality will produce an ordinary ratio at birth, whereas in conjunction with a high mortality it will produce an abnormally low ratio at birth (Parkes(169)).

Variation in the sex-ratio at birth has been described as occurring under a great variety of conditions and circumstances, but in relatively few instances has a substantial case been made out. The remarkable thing about the ratio at birth is its comparative constancy which, as Pike remarks, is maintained under most diverse conditions. Certain small, but apparently well-defined variations do, however, take place and some of the more important of these, together with their probable origins, are discussed below.

B. *Specific variation.*

The sex-ratio varies in different species of mammals and in different races of the same species, notably man. Some of the chief mammals for which data are available are discussed below.

Man. Nichols(147) gives a compilation of 447,019,579 births for all parts of the world. The sex-ratio of this huge number is 105·5, or a male percentage of 51·3. The births in England and Wales during 1838–1914 had an average ratio of 104·0(196). As regards the sex-ratio at birth in different countries, early tables were given by Darwin(45), Ploss(183, 184), Bodio(25), Maurel(139), Bugnion(31), Düsing(58) and others. More recent data have been given by Terry(229), Nichols(147) and, most comprehensive of all, Gini(70). This last author, together with vital statistics returns, may be consulted for actual figures, but as regards Europe it may be said that the occurrence in any wide area of a birth ratio of less than 103 or more than 106 males per 100 females is rare (or was before the war). In North America similar ratios are found.

The Jews, as a race, seem to have been credited with a high percentage of male births and there does seem to be some grounds for supposing that this race has an abnormally large excess of males at birth. The reason is not clear, but may be connected with a decreased pre-natal mortality (for table and discussion see Parkes(169)).

For Eastern races data are less abundant. Newcomb(145) says that in Japan the sex-ratio of more than a million births was much the same as the European, while Bugnion(31) gives the figure for Japan in 1895–1905 as 104·6, which is raised to 104·94 on including the still-births.

Negro races appear on the whole to have a lower percentage of males at birth than do whites, even when living in the same districts. The following table sum-

marises some of the material relating to this point (see also Parkes (153) and Thomas (231)).

Table XXI. *Sex-ratio for white and coloured races living in same locality.*

Authority	Locality	Ratio for whites	Ratio for coloured races
Heape (87)	Cuba	108·42	101·2
Little (126)	U.S.A. (1st births)	115·51	93·61
Nichols (147)	Columbia	106·2	103·0
Jastrzebski (102)	Cape Colony	105·4	102·6
,,	U.S.A.	105·7	100·0 (just under)
,,	New York	104·5	101·6
,,	New Orleans	102·0	98·2
,,	Columbia	105·0	100·0

Scattered data, some of which will be referred to later, exist for a number of native tribes. The sex-ratios of African tribes have been dealt with in detail by Malcolm (135). The dominant feature of these seems to be a variable excess of females at birth.

Asiatic races appear to present similar proportions of the sexes to Europeans, though the following table (quoted from Jastrzebski (102)) showing the ratios for various Indian races shows one or two fairly high male proportions.

Table XXII. *Racial sex-ratio, India* (Jastrzebski).

Province	Representative racial division	Number of births	Males to 1000 females
Behar and Orissa	Mongolo-Dravidian	3,300,000	1040
Coorg	Scythio-Dravidian	14,000	1040
Madras	Dravidian	3,750,000	1045
Central and Berar	,,	2,000,000	1046
Assam	Mongoloid	600,000	1070
United Provinces	Ayro-Dravidian	6,375,000	1082
Punjab	Indo-Aryan	2,600,000	1097
North-west Frontier	Turco-Iranian	225,000	1236

Other mammals. The sex-ratios given by various authors for various domestic animals may best be summarised in tabular form (Table XXIII).

Without exception these statistics are founded on herd and stud book returns, and they cannot therefore be very accurate. Nevertheless, so far as they go, they suggest that the horse and the sheep have the lowest ratio and the dog the highest, while cows and pigs occupy an intermediate position.

Much more reliable data, though on smaller numbers of animals, are available for laboratory animals (Tables XXIV and XXV).

Table XXIII. *Specific variation in sex-ratio of domestic animals.*

Species	Number of births	Males per 100 females	Male percentage	Author
Horse	25,560	99·7	—	Darwin (45)
	84,258	99·65	—	Heyer, quoted by Günther (78)
	708,410	98·03	—	Düsing (59)
	135,886	96·5	—	Düsing (59)
	16,091	97·3	—	Wilckens (243)
	—	96·57	—	Goehlert (71)
Cow	8,179	98·1	—	Heyer, quoted by Günther (78)
	4,900	107·3	—	Wilckens (243)
	480	113·3	—	Pearl and Parshley (177)
	1,313	—	50·0	Pearl (173)
	982	94·4	—	Darwin (45)
Sheep	59,650	97·7	—	Darwin (45)
	6,751	97·4	—	Wilckens (243)
Pig	2,357	111·8	—	Wilckens (243)
	5,970	102·5	—	Parker and Bullard (151)
	3,464	102·7	—	Machens (133)
	16,233	—	48·84	Parkes (156)
Dog (Collie)	6,878	110·7	—	Darwin (45)
(Greyhound)	6,777	118·19	—	Heape (86)
	17,838	118·5	—	Heape (86)

Table XXIV. *Sex-ratio in the rat.*

Sub-species	Number of young	Males per 100 females	Male percentage	Author
Albino	255	105·6	—	Cuénot (43)
	452	107·33	—	King (106)
	1089	107·5	—	King and Stotsenburg (110)
	2818	104·6	—	King (107)
	4992	105·2	2·00	—	King (109)
	944	108·3	—	Slonaker and Card (218)
Extracted albinos	1598	105·6 ± 3·58	—	King (109)
Norway	51	82·1	—	Miller (143)
	1862	85·8 ± 2·68	—	King (109)
Extracted Norways	1740	106·9 ± 3·46	—	King (109)

From these results of laboratory breeding it seems fairly safe to say that the albino rat has a slight excess of males at birth. The Norway rat, on the other hand, seems, when bred in captivity, to have a fairly large excess of females.

Considerable data also exist for the mouse. Schultze (214) found approximate equality in over 1000 mouse births, and Yerkes (249) reports the same for waltzing mice. Copeman and Parsons (40) say that the number of males born is slightly in excess of that of females.

Table XXV. *Sex-ratio in the mouse.*

Sub-species	Number of young	Males per 100 females	Male percentage	Author
Mus musc.	700	100·0	—	Gates, quoted by Günther (78)
Albino	1469	79·80	44·38	Bluhm (24)
(1921–2)	1031	—	54·2 ± 1·04	Parkes (159)
(1922–5)	1872	—	51·7 ± ·77	Parkes (167)
	—	—	50·5	Weldon (239)
Peromyscus	4652	97·37 ± 1·93	—	Sumner, McDaniel and Huestis (227)

C. *Hybridisation.*

As regards the human subject, three types of hybridisation are possible: hybridisation between white races, hybridisation between coloured races, and white and coloured crosses. As a result of a careful investigation of the effect of hybridisation, Little (126) came to the conclusion that hybrid white matings give a significant excess of males over pure white mating, and that hybrid coloured matings give a significant excess of females over pure coloured matings. His actual figures taken from hospital records in New York were as follows:

Table XXVI. *Effect of hybridisation on sex-ratio* (Little (126)).

Mating	Males	Females	Ratio
(a) European pure	2807	2689	105·54 ± 0·97
(b) ,, hybrid	677	551	122·86 ± 2·14
(c) U.S. pure	994	840	118·33 ± 1·71
(d) British West Indies coloured	667	618	107·92 ± 2·65
(e) U.S. coloured	695	723	96·12 ± 1·76

These statistics confirm and amplify an earlier investigation of Little's (125) in which he found that 5753 "pure" stock births gave a sex-ratio of 106·27 ± 1·81 and 1305 "hybrid" stock births gave one of 121·56 ± 4·49.

These conclusions have been confirmed in principle by Lewis (121), who found that unions of Spanish, Italian and French male emigrants with native-born Argentine females produce a higher masculinity than pure Argentine alliances or pure alliances of any of these nationalities in Buenos Ayres. Also, unions of Argentine males with females of foreign nationalities gave a higher sex-ratio than pure Argentine matings. Pearl (176) came to a similar conclusion from similar material. Jastrzebski (102), however, failed to confirm the supposition that hybrid white mating gave an excess of males greater than that found in either parent stock. In the case of coloured and white race hybrids it is stated by Powers (185) that there is a large excess of girls among half-breeds in California, and Kohl (112) notes that in the northern parts of the United States females preponderate in the progeny

of French men with Indian women. In another human hybrid, the mulatto, Starkweather [222] found 12–15 per cent. excess of females, while in the whole population males were in excess. Görtz [73] reports an excess of females among the offspring of Dutch men and Malay women in Java, a fact which has been confirmed by Waitz [238]. Jastrzcbski states that in New York city he found for the years 1910–15 the following ratios of males to 100 females: white 104·0, negroes 99·9, and mulattoes 97·9. Bugnion [31] also found this same type of result.

In so far as this diverse material relating to the human subject can be welded into a generalisation, it is that (a) crosses between white races produce an excess of males over pure white mating, (b) hybridisation between coloured races produces an excess of females above pure coloured matings, (c) hybrids of white and coloured races show an excess of females above the pure matings of either race.

The data for mammals other than man are extremely rare, which is somewhat peculiar, as a large amount of experimental hybridisation has been done recently. King's [106] analysis of von Guaita's [75] material showed that the cross between albino mice and Japanese waltzing mice gave a sex-ratio of 113·17 in 356 individuals. This ratio is probably above that for pure matings of either species. In the cross between the wild Norway rat and the albino rat, King [106] found in 425 individuals 231 males and 194 females, a sex-ratio of 119·07, as against the normal of 105·5 for the albino rat.

In sub-specific crosses of *Peromyscus* Sumner and his collaborators [227] found the following sex-ratios:

Table XXVII. *Sex-ratio in* Peromyscus *species hybrids.*

	Males	Females	Ratio
Pure	1414	1516	93·27 ± 2·32
Hybrid	881	841	104·76 ± 3·41

It is thus tolerably certain that hybridisation may affect the sex-ratio, but it seems difficult to suggest any explanation of this phenomenon.

In one case, however, of an abnormal sex-ratio arising as the result of extreme hybridisation it is certain that a sexually differential elimination of foetuses is the explanation. This is the case mentioned by Babcock and Clausen [6] of the bison and cow cross, which almost always gives rise to females. It appears that a cow is usually physically incapable of bearing a male bison-cow foetus to full time, owing to the uterus of the cow being totally unsuited to the peculiar shape of the male of the cross. A large majority of the males are thus weeded out before birth.

D. *Inbreeding.*

Inbreeding has long been considered to be detrimental to the organism, and both fertility and the sex-ratio have been included among the characters altered by consanguineous mating. Much of the supposed evidence is, however, clearly inadmissible, and consideration is made difficult by the lack of any general accept-

ance of where inbreeding begins and where it ends. In man, for instance, marriages between cousins are usually called inbreeding, whereas in domestic animals the term usually implies brother and sister, or parents and progeny, matings.

East and Jones [61] suggest a means of measuring the intensity of inbreeding based on the number of ancestors an individual can have in any given generation back. Outbred individuals have 2^n ancestors in any given generation back, where n stands for the number of generations back. An animal rising from series of brother and sister matings, on the other hand, never has more than two in any ancestral generation. The above standard of measurement is of course inapplicable to communities, and the data on which the effect of inbreeding in man can be considered consist largely of an unconnected series of anthropological observations.

Düsing [58], arguing from births in isolated communities in which inbreeding might be supposed to take place, came to the conclusion that the result was an excessive proportion of males. He also argues that incest is inversely proportional to the number of males, because the greater the number of males the further from home they have to go to find a female, and further that nature tends to correct shortages of males by making incestuous unions produce an excess of males.

Westermarck [241] concludes that inbreeding raises the sex-ratio, and adds that the sex-ratio is higher in country districts, where more inbreeding goes on, than in towns. Arner [3] discusses the whole subject without coming to any definite decision.

Small islands have been a fruitful source of communities said to be inbred. Lewis [121] found at Port Blair, in the Andaman Islands, that there were among the locally born population 461 male children to only 376 female. Female infanticide is unknown and, owing to the shortage, females are as much desired as the males, and he attributed the peculiar sex-ratio to inbreeding. In Nicabar Lewis again found an excess of males, and only brother and sister matings are prohibited. A census [251] of seven islands belonging to the Duke of York group showed that between September, 1898, and May, 1900, 131 boys and 122 girls were born, a ratio of 107·3. All marriages were between relatives. Huth [98], however, gives a long list of consanguineous communities where the sex-ratio was undisturbed.

Such contradictory and inconclusive evidence as this is only slightly less unsatisfactory than that existing for domestic animals. Goehlert's [71] statistical investigation on horses is of no value because his criterion of consanguinity, namely, similarity of coat-colour, is manifestly absurd. Bell [12] says that in the inbred herd of Bate's Shorthorns at Kirklevington, the bull calves greatly exceed the heifers, and the same is reported by Carr [34] for the inbred Shorthorns at Warlaby. Heape [86] ascribes to inbreeding the high sex-ratio of many thoroughbred dogs. Beaudouin [10] gives precisely contradictory data.

Turning to laboratory animals, various observations have been made. Schultze [214] came to the conclusion that inbreeding had no effect on the sex-ratio, although he got a ratio slightly higher than the normal. Huth [98] found in rabbits a low ratio as the effect of inbreeding, and Copeman and Parsons [40] obtained a similar result in mice.

Probably the only entirely reliable and extensive data on the effects of inbreeding on the sex-ratio are provided by the work of King (107) on rats. This author found in 3256 young, produced as the result of brother and sister matings for six consecutive generations without selection, a sex-ratio of 108·6, against the normal of 105·0. In the earlier generations the animals all suffered from malnutrition, and King came to negative conclusions. If brother and sister matings on the scale carried out by King have no effect on the sex-ratio it seems fairly certain that the much diluted form of cousin matings which passes for inbreeding in man would probably not produce a pronounced effect.

So far as can be judged, therefore, no indisputable evidence exists that inbreeding affects the sex-ratio: a conclusion which is also arrived at by East and Jones.

E. *Annual variation.*

Man. In England and Wales the variation in the sex-ratio at birth between 1838 and 1914 was between 105·4 in 1843 and 103·2 in 1898. The average of 104·0 is nearer the lower figure. Since 1914, however, the proportions of males has risen appreciably (196). Similar data are available relating to other countries, and a wealth of statistical material is to be found in Gini's (70) comprehensive book. Annual variation in the ratio at birth in countries of fairly stable conditions is comparatively small, and in any case its biological interest appears to be slight. At the same time reference may be made to a diagram in a recent issue (1919) of the Registrar General's reports where annual variation in the sex-ratio for the last half-century is plotted with the variation in the *Economist* index price of food. There is a general resemblance between the two curves, increase of male proportion being roughly correlated with rises in price of food. The correlation is, however, only very general, and the similarity between the two curves is greatly accentuated by the sudden rise in both during the war. Whether this apparent correlation has any biological meaning is difficult to say, but the idea is strongly reminiscent of Düsing's (58) endeavour to correlate the annual variation in the birth ratio in Prussia between 1749–1849 with the success or failure of the harvests. (In this connection see also Gini (70).)

Other mammals. Annual variation in the sex-ratio at birth appears to be pronounced in the domestic animals, but accurate data are generally lacking. Heape's (86) figures for dogs show extremes of 111·1 and 121·17 males per 100 females during the years 1886–92.

For laboratory mammals practically no material exists. King's (110) rats showed a sex-ratio of 106·9 during 1911–13 (275 litters) and one of 108·1 in 1914 (814 litters); the male percentage in mice for four consecutive years was found by the present writer (167) to be 54·2 ± 1·04, 50·4 + 3·22, 52·2 ± 1·42, 51·4 + 1·09. No one of these percentages is significantly different from any other. As regards laboratory animals, however, it should be emphasised that considerable seasonal variation in the sex-ratio has been demonstrated in many cases, and that, therefore, the annual ratio may be an arbitrary figure depending on the relative number of births in each season.

F. *Seasonal variation.*

Though no definite breeding season can now be observed in civilised man it is generally assumed that the human species originally had a primitive breeding season. This assumption is supported by the fact that in some countries considerable seasonal variation in the birth-rate is found, while in some aboriginal peoples breeding appears to be entirely restricted to certain seasons. Wild mammals, of course, normally have a quite definite breeding season. In view of these considerations, many attempts have been made to demonstrate seasonal variation of the sex-ratio in man, and in domestic and laboratory animals.

Man. Düsing[58] brought forward data for Prussia, 1872–81, which seemed to show that the slight seasonal variation in the sex-ratio was connected with the slight seasonal variation in the conception rate, lowness of conception rate being connected with the higher proportions of males. Since, however, the monthly percentages of total conceptions for the year varied only between 8·8 and 7·6, while the males per 100 females varied only between 106·77 and 105·92, it seems difficult to attach much importance to the figures. The illegitimacy figures alone, however, showed greater variation and the same correlation. Heape's[87] data for Cuba, summarised below, show much the same thing:

Table XXVIII. *Birth-rate and sex-ratio of Cuba, 1904–6*
(*collected from* Heape*).*

Race	Sex-ratio of births in months of highest fertility	Sex-ratio of births in months of lowest fertility	Sex-ratio for whole year
Whites	104·29	108·21	107·14
Coloured	99 3	108·3	100·07

Since a very definite periodicity exists in the birth-rate in both blacks and whites, these figures are rather striking. Similar views have been expressed by Goehlert[72] and Sormani[219].

Bonnier[27] criticises Heape's conclusions primarily on the grounds that Heape maintained that his figures showed that the ovum was instrumental in determining sex. It should be pointed out, however, that Heape's actual data may be perfectly valid and yet be in keeping with present views of sex-determination. Bonnier's own statistics for Sweden, however, show that while a certain periodicity in the birth-rate is found, no correlated variation in the sex-ratio exists. As regards the actual means whereby seasonal variation in the ratio at birth might be brought about, Bonnier considers that a seasonal variation in still-births would be sufficient to produce the result, but, as I have pointed out elsewhere[166], there are grave objections to such a view, and it seems more probable that seasonal variation of the ratio at birth in man will be traced back to conception.

Pig. Machens[133] was unable to demonstrate any regular seasonal variation

in the sex-ratio of 3464 pigs, and a similar negative result was obtained by the present writer (166) from the records of 10,961 pigs. In view of the highly domesticated nature of the pig these results are not really surprising.

Dog. Heape (86) appears to have shown some connection between sex-ratio and birth-rate in collie dogs and greyhounds, but the connection is not very clear, and in the case of the greyhounds, the most striking correlations are founded on very small numbers.

Rat. King's (110) breeding experiments on rats show definite seasonal variation in the sex-ratio. The actual figures are as follows:

Table XXIX. *Seasonal sex-ratio in rats*
(collected from King).

Seasons	Sex-ratio		
	1911–13	1914	1911–14
March–May	94·2	103·8	99·0
June–August	119·9	115·6	117·7
Sept.–Nov.	104·4	106·2	105·3
Dec.–Feb.	111·6	99·0	105·3
Totals	106·9	108·1	107·5

Litters cast in the spring, therefore, have an appreciably lower ratio of males than those produced in summer. Hanson and Sholes (82), however, failed to find seasonal variation in either sex-ratio or fertility.

Mouse. In *Peromyscus* Sumner, McDaniel and Huestis (227) found considerable seasonal variation in the proportion of males, which was fairly constant from year to year. Their summary figures are:

Table XXX. *Seasonal sex-ratio in* Peromyscus.

Season	Sex-ratio and probable error
Feb.–April	104·23 ± 3·85
May–July	91·48 ± 3·48
Aug.–Oct.	102·29 ± 3·68
Nov.–Jan.	85·21 ± 4·62

These figures show a much more definite biennial rhythm than do King's, and also the periods of low male proportion are at a rather different time, but there is no reason why the rat and *Peromyscus* should show identical seasonal variation. The undoubted fact is that both do show variation.

In the albino mouse, the present writer (159, 167) has found considerable evidence of seasonal variation in the male percentage.

Table XXXI. *Seasonal variation in sex-ratio in mice.*

Season	Sex-ratio	
	1921–2	1922–5
Oct.–Dec.	—	55·9 ± 1·83
Jan.–March	—	51·9 ± 2·10
April–June	49·9 ± 1·77	48·2 ± 1·46
July–Sept.	56·1 ± 1·38	52·2 ± 1·23
Total	54·2 ± 1·04	51·7 ± ·77

These figures show that during the early summer the proportion of males is appreciably less than during the autumn.

The exact reason for this seasonal variation in laboratory animals is not apparent, but there is evidence which makes it probable that some temperature factor is at work, and in addition it seems necessary to suppose that the variation originates at conception. If variation in pre-natal mortality is to account for the fact (*i.e.* low sex-ratio in middle of breeding season) it would be necessary to suppose the amount of pre-natal mortality increased during the optimum breeding season, and it can hardly be supposed that this takes place, because reproduction presumably takes place under optimum conditions at that time. There appear to be no actual data relating to seasonal variation in pre-natal mortality in any mammal (excepting some still-birth data for man).

G. *Age of parents.*

That the sex-ratio varies according to the age of the parents is one of the oldest of all observations on the sex-ratio, and variation has been described by various authors according to (*a*) the age of the mother, (*b*) the age of the father, and (*c*) the relative age of the parents.

Hoffacker [94, 95] produced statistics showing that old and young mothers had a greater excess of males than had middle-aged mothers. Figures of similar import were given by Bidder [20] and Düsing [58], while Hampe's [81] figures show no very regular tendency. Rosenfeld [200] came to the conclusion that the excess of males declines with increasing age of the mother, a finding supported by figures obtained by the present writer [158], by Brewster [29] and by Specht [220]. Similar data are also presented by Stadler [221], Kollmann [113] and Boudin [28]. The present writer has also shown that pre-natal mortality increases very considerably with increasing age of the mother [158], and this probably accounts for the influence of the age of the mother on the sex-ratio at birth. If this explanation is correct the fact that the age of the mother has an influence on the sex-ratio implies no contradiction to the chromosome theory of sex-determination.

As regards laboratory mammals, King [110] says that the sex-ratio in rats varies with the age of the mother, but the actual criterion used is the number of the pregnancy (discussed later). Data for the mouse, quoted by King from Copeman

and Parsons (40), show sex-ratios for conceptions at 2, 3·5 and 6 months of 103·7, 96·5, 123·3 respectively, but the numbers are meagre.

The influence of the age of the father has been considered by various authors, Hoffacker (94, 95), Rosenfeld (200), Francke (65) and Dumont (56), but no very consistent data are available. There is, in fact, no very definite evidence for supposing that the age of the father has any weight. Since parents generally tend to be of not very different ages, any influence exerted by the age of one parent would appear to some degree when the age of the other was considered.

Such considerations as this have been the basis of a large number of practically valueless statistical investigations on the influence of the relative age of the parents (Hoffacker (94); Sadler (208); Gochlert (72); Stieda (224); Berner (14); Kollmann (113)). The results of these several investigations are summarised in Geddes and Thomson's book (69) and further notice need not be paid here, except to say that the results are all mutually contradictory. Methorst (140) concludes that the relative age of the parents has no significance.

II. *Number of the pregnancy.*

It is clear that a loose connection exists between the age of the mother and the number of the pregnancy. First births, for instance, normally take place between 20-30 years of age, whereas third, fourth and later births are usually after that age. Some statistics discussed elsewhere by the present writer (162) showed two-thirds of the primiparae to be under 28 years of age. Second and third births were concentrated between 23 and 32 years of age, while fourth births were largely between 28 and 37 years of age. Later births were mostly from mothers of over 32 years. These were hospital statistics and therefore mostly relating to a class of people who breed earlier and often, but the connection between number of pregnancy and age of mother is obviously of general application. This means that if the age of mother influences the sex-ratio, then the same variation should be apparent in the sex-ratio of different chronological births, or *vice versa*. At present there seems good reason to suppose that in the same way as the male proportion decreases with advancing

Table XXXII. *Sex-ratio according to number of pregnancy (human).*

Number of pregnancy	Author and material			
	Punnett (191)	Punnett (191)	Punnett (191)	Parkes (158)
	Burke's Peerage	Torres Straits	Murray Island	Hospital
1	140·0	113·4	120·9	115·0
2	117·2	110·4	114·5	100·9
3 (+)	104·1	95·9	102·2	115·0
4 (+)	102·6	93·3	—	105·9
5	101·2	—	—	115·0
6				
7	102·7	·	—	
8 (+)				97·2
9	100·0	—	—	—

age of mother, the proportion also decreases as the number of the birth becomes higher.

Newcomb (145) came to not dissimilar conclusions.

It is generally recognised that first births have a very high sex-ratio. Ahlfield (2) puts the ratio at 137, Winckel (245) at 136·8, and Hecker (88) at 133·0. Little (126) found from the records of the Soloane Maternity Hospital that European pure matings gave a sex-ratio of 115·51 for first births and one of 97·33 for subsequent births.

It seems possible to say, therefore, that the male percentage decreases both with advancing age of the mother and with multiparity, and that since the amount of pre-natal mortality increases with both factors (158), the result is probably brought about by increased pre-natal elimination of males. On account of the casual relation between the two it seems almost impossible to say whether the age of the mother or the number of the pregnancy (if not both) is the factor governing the amount of intra-uterine death.

As regards other mammals very little data are available. Both King (110) for rats and Copeman and Parsons (40) for mice agree, however, that the male percentage decreases after the first litter. The following is King's table for rats:

Table XXXIII. *Sex-ratio according to number of litter.*

Order of litter	Number of litters	Number of individuals	Males	Females	Sex-ratio
1st	116	717	385	332	115·9
2nd	116	843	426	417	102·2
3rd	103	671	328	343	95 6
4th	89	587	302	285	105 9
Total	424	2818	1441	1377	104 6

I. *Size of litter.*

In the first place it is necessary to differentiate between multiple births in animals which are normally monotocous and the litters of normally polytocous animals.

As regards twins in the human subject, the gross sex-ratio is reported by Pearl (175) to be slightly below that for normal births. In the case of triplets Pearl found the remarkably low ratio of 54·8 males per 100 females. Duncker (57), however, quotes figures which suggest nothing abnormal in the gross sex-ratio of twins and triplets, the respective ratios for 1000 of each being 104·8 and 107·7. This finding is supported by Newcomb (145) for human twins and by Wentworth (240) who found in 146 cases of sheep triplets a ratio of 106·5. Bertillon (16) found a ratio of 101·5 among human twins. The weight of the evidence, therefore, seems to show that human multiple births do not show any very abnormal gross sex-ratio. The peculiarity of the sex constitution of these multiple births will be considered later.

Turning to normally polytocous animals, many investigations of the effect of litter size are on record. The results, however, are hopelessly contradictory. The following table summarises the chief of these studies:

Table XXXIV. *Sex-ratio according to litter size.*

Litter size	Sex-ratio, author and species						
	Pig			Rat	Mouse		
	Parker and Bullard (151)	Machens (133)	Parkes (155)	King (110)	Sumner (227)	Parkes (159)	Parkes (167)
1	150·0	—	—		104·9		
2	117·5	—	—		92 1		
3	98·2	—	—	100·7	95·0	83·7	106·5
4	117·0	—	131·0		101·5		
5	93·7	100·0	134·8		103·1		
6	104·0	92·8	105·0		95·6	120·5	111·6
7	113·4	96·4	112·0	110·6			
8	106·7	100·0	111·5		164·7		—
9 (+)	96·1	96·9	99·0	106·8			
10	86·0	102·6	100·6	—	—	125·4	102·0
11	88 8	96·5	105·0	—	—		
12 (+)	104·7	103 5	124·5	—	—		
Total	102 5	102·7	107·3	—	—	—	—

The size of a litter depends on two factors: the number of ova fertilised and the amount of pre-natal mortality. Of these two factors the first can hardly have any effect on the sex-ratio, whereas the second is well known to influence the ratio at birth. Since then the sex-ratio depends on two factors, one of which influences the ratio at birth, and one of which does not, it is not surprising that the results are incoherent. Until further information is available relating to the relative importance of the two factors in moulding the size of litter, little good can come of further discussion.

J. *Polygamy.*

Data for polyandrous mating do not appear to be available, but certain information is to be found with reference to the effect of polygyny on the sex-ratio.

Most of the evidence relating to polygyny is anthropological in nature, and is largely contradictory. Even the vital statistics of the Mormons have been invoked to demonstrate the effect of polygyny. Thus Burton [32] reported a large excess of females among the Mormons, but this was later contradicted by Newcomb [145]. Sanderson [210] found nothing abnormal in the sex-ratio of the polygynous Kaffirs of Natal, and Campbell [33] considered 440 births from 17 polygynous men in Siam and found a sex-ratio of 108·5, which cannot be supposed to be abnormal. Thomas [230], however, found the following figures for the Ibo of the Auka:

Table XXXV. *Polygyny and sex-ratio.*

Number of wives	Percentage of males
1	49
2	51
3	52
4	55
5 +	57

The degree of polygyny found in man, however, is clearly very slight compared with that common among farm animals, and it might be expected, therefore, that the latter would show any effect much more clearly. As I have pointed out elsewhere, however, farm animals have no very abnormal sex-ratios, and sheep, in particular the most polygynous of farm animals, tend even to have a low one. Düsing(59), however, gives the following table showing the sex-ratio of foals produced by stallions according to the number of times to stud in a season.

Table XXXVI. *Amount of stud work and sex-ratio in horses* (Düsing).

Number of times to stud	Number of foals		Sex-ratio
	Males	Females	
60–	71,407	70,569	101·19
55–59	75,493	74,912	100·77
50 ·54	69,972	71,461	97·92
45–49	69,774	72,073	96·81
40–44	66,573	69,045	96·42
35–39	44,911	46,493	96·60
20–34	29,023	29,934	96·94
Total	427,153	434,487	98 31

In the mouse I have obtained some data tending to agree with Düsing that increasing the degree of polygyny increases the male proportion(164), but any generalisation is clearly impossible at the present time.

K. *Nutrition.*

The study of the effect of nutrition on the sex-ratio was, until comparatively recently, inextricably bound up with the idea that sex was determined during gestation. Thus all kinds of observations are on record tending to show the excess production of one sex or the other under good or bad conditions, and until 30 years ago the current literature on the sex-ratio consisted of notes such as: "Une tribu arabe capture en Egypte plusieurs centaines de femmes. 482 deviennent enceintes pendant la marche de la caravanne. Résultat: 79 ♂s et 403 ♀s" (D'Oranovskaia (148)).

Punnett(191) graded the population of London into three classes, according to social status and probable nutrition, and found a higher proportion of males in

the better-fed classes, a result which was contrary to previous ideas of the influence of nutrition. In this connection the statistics (mentioned above) tending to show that the annual variation in the sex-ratio in man is connected with the availability of food should be remembered.

Schultze(214) conducted very vigorous experiments into the effect of mal-nutrition in mice, but obtained inconclusive results. (For further discussion see Parkes(160).)

Recently, Slonaker and Card(218) have reported lowering of the sex-ratio in rats by restriction of diet. The control animals gave a ratio of 108·0, while young from groups on restricted diets had an excess of females, the ratio for one group being as low as 84·0. The numbers, however, are small and of doubtful statistical significance.

The effect of vitamin B deficiency of male rats has been considered by Parkes and Drummond(170), the general conclusion being that decreasing the vitamin B supply decreases the proportion of males. The same authors failed to find any effect on the sex-ratio of previous vitamin A deficiency(171).

L. Sex-combinations in multiple births.

In multiple births the sex may occur in various combinations. Thus twins may be both females, both males, or one male and one female. In triplets there are four possible combinations (3 ♀, 3 ♂, 2 ♂ : 1 ♀, 2 ♀ : 1 ♂), and in larger multiple births the possible combinations are of course numerous, being in each case one more than the size of litter. If the distribution of the sexes were a matter of chance the various possible combinations should appear in a certain calculable frequency, and the theoretical chance distribution may be compared with that actually found. At the outset it is necessary to distinguish between multiple births in mammals which are normally monotocous and cases where multiple birth is normal. In the former case an appreciable proportion of the multiple births are due to polyembryony, and the unisexuality of monozygotic twins and triplets makes the sex distribution definitely not a matter of chance. The difference between the theoretical chance expectation and the actual distribution of the sexes in twins and triplets has indeed been used to calculate the frequency of polyembryony. Thus in 1000 twins and 1000 triplets Duncker(57) found the following distribution:

Table XXXVII. *Actual and probable sex-combinations*
in human multiple births.

Number of males	Twins		Triplets	
	Actual	Probable	Actual	Probable
	—	—	245	137·9
	326	261·7	285	387·1
	371	499·6	245	362·1
	303	238·7	225	112·9

This table shows that unisexual births are about twice as numerous as they should be on a chance basis, and suggests that about one-third of all twins and one-quarter of all triplets are monozygotic. Similar figures are given by Nichols[147] and by Gini[70].

Bertillon[16] gives some interesting figures showing the variation of the sex-constitution of twins at different ages of mother:

Table XXXVIII. *Sex-combinations in twins according to age of mother.*

Age of mother	Per 1000 twins at each age group		
	2 ♂s	2 ♀s	1 ♂, 1 ♀
18–25	348	382	270
26–35	343	302	355
36–45	348	320	332
46 +	314	326	360

Since the excess of unisexual births is least with old mothers and most with young mothers it may be supposed that polyembryony is more prevalent in young mothers.

Further figures given by Bertillon[16] also suggest that racial variations in the amount of polyembryony occur:

Table XXXIX. *Racial variation in polyembryony.*

Country	No. per 1000 pregnancies	Sexes per 100 twins	
		2 ♂s, 2 ♀s	1 ♂, 1 ♀
France	10·0	65 1	34·9
Italy	10·36	64 3	35·7
Prussia	12·50	62·5	37·5
Austria	11·90	62 0	38·0
Hungary	13·0	61·3	38·7

Since the author gives the theoretical percentage expectation of unisexual births at 50·05, the amount of polyembryony is clearly appreciable in all cases, but is 30 per cent. more in the case of France than in the case of Hungary. Bertillon's figures for triplets also suggest the same conclusions.

For multiple births in normally monotocous farm animals little data appear to be available. Wentworth[240] found nothing unexpected in the distribution of the sexes in sheep twins, and Lillie[122], on cattle, was unable to arrive at any definite conclusions.

In normally polytocous animals, polyembryony apparently plays but little part in moulding the size of the litter, so that statistics for such animals may be considered to be reasonably free from this complication.

In the *Peromyscus* Sumner, McDaniel and Huestis[227] found that the observed

frequencies of the various possible combinations of the sexes in the various litter sizes were very close to the theoretical expectation. In the pig, however, the present writer (156) found that litters having fairly equal proportions of the sexes were more frequent than the expectation. The following table sums up the data (the classes relate to the number of males in the litters): Central classes, approximately equal numbers of sexes; Lower extremes, mostly females; Upper extremes, mostly males.

Table XL.

Class	Number of litters		Difference
	Observed	Calculated	
Lower extremes	66	90·4	− 24·4
Lower intermediates	520	551·2	− 31·2
Central classes	890	774·4	+ 115·6
Upper intermediates	428	477·2	− 49·2
Upper extremes	57	67·4	− 10·4
Totals	1961	1960·6	—

The meaning of these results is difficult to understand.

Finally, this section may be closed with a reference to the numerous investigations which have been made on the sex-combinations in various sizes of human family. Material relating to this subject has been collected and analysed by Venn (237), Newcomb (145), Nichols (146), Gini (70) and the present writer (161). In view of the sociological factors involved, however, the results seem to be of little or no biological importance.

M. *Miscellaneous factors.*

It has long been known that the sex-ratio of illegitimate births shows a lower masculinity than is normal. Darwin (45) notes the fact in considering the sex-ratio in relation to his theory of sexual selection, and comments upon its universal application. Düsing (58) found that the sex-ratio of illegitimate births in Prussia was 105·019, as against a total ratio of 106·28. As these figures were obtained from a very large number of births they were probably thoroughly representative. Bodio (25) gives the sex-ratio of illegitimate births as 104·15. The *Bulletin de l'Institut international de Statistique* gives the average for twenty-seven European countries as: legitimate births, sex-ratio = 105·6; illegitimate births, sex-ratio = 104·2. Prevost (187) gives for sixteen countries an average of 106·06 and of 102·54 respectively. In England and Wales for 1919 the respective figures were 106·0 and 105·2.

The only contrary record seems to be that of Srdinko, who found that, in Austria, the sex-ratio of legitimate births was lower than that of illegitimate births. This, however, he explains by the fact that, whereas the Jews in that country provide but a small fraction of the total births, they provide a large part of the illegitimate births, and, as mentioned above, the Jews have a higher sex-ratio than other European races. For the white races in Cuba, Heape found that the legitimate

sex-ratio for the years 1904–6 averaged 107·78, and the illegitimate 104·4. It is noteworthy that the annual variation in the two classes follows a parallel course. For the coloured races the sex-ratio of legitimate births is 106·76 and that of the illegitimate births 96·76.

This shows that though there is a big difference between the legitimate and illegitimate sex-ratios among the white, the difference is even greater among the coloured. The most probable explanation of this lowering of the sex-ratio is that an increased foetal mortality is set up by the adverse conditions which usually attend illegitimate gestations. It is in fact known that the still-birth rate and infantile mortality rate are much higher in illegitimacy.

Much has been written on the influence of war on the sex-ratio at birth, and many authors claim that an increase of the male percentage takes place during and after war. Newcomb (145), however, found no rise after the American Civil War, and Lehr (119) considers that the fluctuations are within the normal range. Nichols (147), too, found no change for France during 1806–72. Henneberg (90), Ploss (184), Berner (14), Düsing (58) and Savorgnan (211), however, all consider that the male proportion increases. The last author gives the following data for 1914–19:

Table XLI. *Rise in the sex-ratio*, 1914–19.

Year	Sex-ratio	
	England and Wales	Germany
Pre-war average	104·0	105·3–105·9
1915	104·0	105·5
1916	104·9	106·5
1917	104·4	106·9
1918	104·8	107·3
1919	106·0	—

Various hypotheses, ranging from decreased copulation to sex-reversal, have been put forward to explain this increase, but no theory supported by facts appears to have been proposed.

Pitt-Rivers (182) has recently put forward the view that race decline is associated with an increase in the proportion of males, and, conversely, that a stable or increasing population tends to show an excess of females. With our present meagre material, however, the analysis of cause and effect is so difficult that this hypothesis, even if of general applicability, is of small biological interest for the moment.

N. *Experimental modification of the sex-ratio.*

One or two experiments resulting in modification of the sex-ratio may be mentioned here.

Since Pearl (174) showed that persistent alcohol intoxication of the male brought about a selective action among the spermatozoa a number of workers have

endeavoured to bring about alteration of the sex-ratio by means of alcohol and other intoxications.

Bluhm (24) dealt with the effect of alcohol, yohimbin and caffeine on mice. The results were as follows:

Table XLII. *Effects on sex-ratio of alcohol, yohimbin and caffeine* (Bluhm).

Parents	Offspring		Sex-ratio
	Males	Females	
Neither treated	652	817	44·38
Alcohol to male	182	149	54·98
Yohimbin to male	185	154	54·57
Yohimbin to female	112	144	43·75
Caffeine to male	188	162	53·71
Caffeine to female	145	177	45·35

Danforth (44) also found an increase in the male proportion after treatment of the male parent with alcohol. The actual results were as follows:

Table XLIII. *Effect of alcohol intoxication of male* (Danforth).

	Total	Males	Females	Sex-ratio
Males treated	374	210	164	128·0
Males untreated	1132	575	557	103·2

Crew (unpublished observations) failed, however, to effect modification of the ratio by means of alcohol intoxication. The present writer, on the other hand, working in conjunction with C. W. Bellerby, confirmed the results of Bluhm and Danforth in finding an increase in the male proportion. MacDowell and Lord (132), however, obtained only negative results in a long series of experiments.

On the whole it seems permissible to conclude that alcohol intoxication may increase the male percentage. The probable explanation may be considered as an extension of Pearl's original theorem, *i.e.* that alcohol incapacitates the less viable spermatozoa first, and that more X than Y spermatozoa are affected.

Exposure of the male to X-rays in doses insufficient to produce sterility has also been found to modify the sex-ratio (Parkes (168)). The data relating to this are summed up below:

Table XLIV. *Effect of X-rays on sex-ratio.*

Time of conception (days after irradiation of male)	Young		Male percentage
	Males	Females	
0– 4	79	54	59·4 ±2·87
5–18	48	95	33·6 ±2·66
19 +	118	99	54·4 ±2·27

Since 735 normal young bred under precisely similar conditions gave a male percentage of 51.6 ± 1.24, the early excess of males and the later swing over to femininity are striking. The male excess for conceptions within four days of the irradiation is most probably connected with the effect on the spermatozoa already mature at the time of irradiation. It seems difficult to suggest why a different action should be produced on those in the course of formation at the time of irradiation, but it is worthy of note that non-disjunction of the X and Y chromosomes (non-disjunction has been shown to occur as a result of X-irradiation) in a certain number of cases resulting in the formation of spermatozoa of XY constitution would bring about the observed result of female excess during the second period.

VI. THE INHERITANCE OF THE SEX-RATIO.

Until comparatively recently the problem of the inheritance or non-inheritance of the sex-ratio was always treated with the problem of sex-determination and sex-heredity. In reality the problems are quite distinct. Since the dimorphism of the spermatozoa is responsible for the general nature of the sex-ratio, there must always be a hereditary tendency to produce the two sexes in equal numbers and to this extent only have the terms "heredity of sex" and "inheritance of the sex-ratio" a common meaning. The latter term should, however, be confined to the conception that the small deviations from equality which are found in the sex-ratio are subject to hereditary influence.

Very little data are available relating to this point. Biometric workers 20 years ago came to the conclusion that no inheritance was detectable. Since, however, considerable confusion then existed between the problems of sex-determination and the problems of the sex-ratio, and since their work appeared designed to solve both at once, their conclusions are of no very great value. Woods (248), for instance, says: "Thus we may conclude that the determination of sex, in man at least, can be shown to be unaffected by hereditary influence," while Heron (91), in supporting Woods' conclusions, maintains: "Thus on rather wider data, in horse as well as in man, Dr Woods' position is confirmed: there is no inheritance, or at least no sensible inheritance, of sex." These workers were supported by Weldon's data for mice (239).

The problem really comes down to—What factors causing variation in the sex-ratio could be of an inheritable nature? This question is difficult to answer, but from the fact that certain families (Parkes (152)) and certain races have a typical sex-ratio over many generations and in a variety of circumstances, it must be concluded that certain factors, at any rate, causing variation in the sex-ratio may be related to hereditary causes. Davenport (46, 47) considers that the inheritance of twinning through the male is connected with inherited viability of the twin foetuses.

VII. POSSIBILITIES OF CONTROLLING THE SEX-RATIO.

Since something is known of the factors which determine sex, and of the factors which control the sex-ratio, it is possible to point out the paths along which the problem of sex-control must be approached. In the first place it may be said that the aim must be to control the type of individual conceived. The elimination of one sex or other during gestation by death or complete sex-reversal can clearly be discounted as practicable methods of controlling the ratio at birth, and it would therefore be necessary to so control the type of gametes participating in fertilisation as to ensure of the embryo being of the desired sex. Since the type of spermatozoon fertilising the ovum normally determines the sex of the embryo, some kind of control of the type of spermatozoon participating in fertilisation would seem to be the most likely method, but at the same time the ovum is not entirely ruled out as a sex determiner under certain circumstances.

Elimination of one type of spermatozoon after spermatogenesis. It has been pointed out in Section IV that there is good reason to suppose that in most mammals there exists a slight excess of males at conception and that this is due to the greater viability and possibly mobility of the male producing spermatozoa. In addition, various experiments (alcohol intoxication, for instance) have shown fairly definitely that a greater than normal elimination of less viable spermatozoa can be artificially achieved and that this may be to some extent reflected in an increased proportion of males. One possible line is thus presented of materially increasing the percentage of males even if complete elimination of females could not be achieved. It should also be noted that, since even halving the number of spermatozoa available does not affect the capacity of the male to father as many embryos as a female can carry, such a method would not have the undesirable effect of reducing fertility.

Sex-reversal. It seems not improbable that it will be possible at some not distant date to produce mammals experimentally with a sex-differentiation differing from that implied by the chromosome constitution, and the production of such sex-reversals, if functional, in all probability will solve the problem of the control of the sex-ratio. A mammal functioning as a male, for instance, with a chromosome constitution XX (female) would produce spermatozoa of one type only and the progeny resulting from such spermatozoa would, in all probability, be only female. Diagrammatically, the results would be:

Sex-reversal of an XY zygote, on the other hand, would have the following result:

```
        Sex reversed                          Normal
           male                                male
          X  Y                                X  Y
           ↓                                   ↓
        ⎧ X ══════════════════════════════ X ⎫
   Ova  ⎨                    ╳                ⎬  Spermatozoa
        ⎩ Y ══════════════════════════════ Y ⎭
                            ↓
                        Zygotes
                      XX · female
                      2 XY = male
                       YY = ?
```

Since ova as well as spermatozoa would be dimorphic, three types of zygote would result, *i.e.* 1 XX female, 2 XY male, and 1 YY, which would presumably die. Sex-reversal, then, would seem to offer possibilities of producing one-third only females or all females. It is difficult to see how an all male progeny is going to be secured (see Huxley (99)).

The effect on the sex-ratio of non-disjunction. The type of phenomena to be mentioned here has already been described as occurring naturally and has also been experimentally produced in various lower organisms, notably *Drosophila*, and its experimental extension to mammals might be used as some sort of control of sex. If the two X chromosomes of the female fail to become separated during oogenesis an ovum with XX constitution is produced, and fertilisation results in all females (XXY) being produced, XXX organisms dying. Four types of ova are produced by these females, and fertilisation results as follows:

Ova	Sperm	Zygote		Sperm	Zygote
XX	Y———————XXY (♀)		X———————XXX (dies)
XY	Y——————— XYY (♂)		X———————XXY (♀)
X	Y——————XY (♂)		X———————XX (♀)
Y	Y———————YY (dies)		X———————XY (♂)

The net result, therefore, is that, since fertilisation will be achieved by X and Y spermatozoa more or less equally, the two sexes will be produced in equal numbers.

Non-disjunction in spermatogenesis, resulting in XY spermatozoa, would produce XXY (female) individuals which would presumably have the same effect as the XXY females discussed in the preceding paragraph.

BIBLIOGRAPHY.

(1) AHLFIELD (1872). "Die Geburten älterer Erstgeschwangester." *Arch. f. Gyn.* **4.**
(2) —— (1876). "Ueber den Knabenuberschuss älterer Erstegebärenden." *Arch. f. Gyn.* **9.**
(3) ARNER (1908). *Consanguineous Marriages in the American Population.* New York.
(4) ASHBY (1915). *Infant Mortality.* Cambridge.
(5) AUERBACH (1912). "Das wahre Geschlechtsverhältnis des Menschen." *Arch. f. Rassen- und Gesellschafts-Biol.* **9.**
(6) BABCOCK and CLAUSEN (1918). *Genetics in Relation to Agriculture.*

(7) Bälz (1911). "Die Verhältniszahl der Geburten in verschiedenen Ländern." *Korrespondenzbl. deutsch. Anthrop. Ges.* **42**.

(8) Basile (1908). "Influenza della Lecitina sulla determinazione del sesso e sui caratteri mendeliani." *Atti Accad. Lincei*, **27**.

(9) Baust (1871). *Die Ursachen welche die Entwicklung des männlichen und weiblichen Geschlechts bedingen.* Stuttgart.

(10) Beaudouin (1862). "Faits pour servir à l'histoire des effets de la consanguinité chez les animaux domestiques." *Compte Rendu Acad. Sci.* **55**.

(11) Bell, A. G. (1914). "Sex determination in sheep." *Journ. Hered.* **5**.

(12) Bell, T. (1871). *The History of the Improved Shorthorn, or Durham Cattle.* Newcastle.

(13) Bentzen (1860). "Ueber der Sterblichkeit im ersten Lebenjahr." *Schmidt's Jahrb.* **108**.

(14) Berner (1883). *Om kjönsdannelsens Aarsager. En biologisk studie.* Christiania.

(15) Bernouli (1841). *Handbuch der Populationstatistik.*

(16) Bertillon (1875). "Des combinaisons du sexe dans les grossesses gémellaires." *Journ. Soc. Stat. Paris*, **16**.

(17) —— (1893). "De la mortalité avant la naissance." *Journ. Soc. Stat. Paris*, **34**.

(18) —— (1896). "De la mortinatalité et des naissances prématurées selon l'âge du foetus et selon l'âge de la mère." *Revue d'hygiène*, **18**.

(19) —— (1898). "La gémellité selon l'âge de la mère et le rang chronologique de l'accouchement." *Journ. Soc. Stat. Paris*, **39**.

(20) Bidder (1878). "Ueber den Einfluss des Alters der Mutter auf das Geschlecht des Kindes." *Zeits. f. Geburts. und Gynäk.* **2**.

(21) Biedl, Peters and Hofstatter (1921). "Experimentelle Studien uber die Einnistung und Weiterentwickelung des Eies im Uterus." *Zeits. f. Geburts. u. Gynäk.* **84**.

(22) Bluhm (1912). "Zur Frage nach der generativen Tuchtigkeit der deutschen Frauen und der rassenhygienischen Bedeutung der ärztlichen Geburtshilfe." *Arch. f. Rassen- und Gesellschafts-Biol.* **9**.

(23) —— (1918). "Zur Kenntnis der Gattungsleistungen der Industriearbeiterinnen im Kriege." *Arch. f. Rassen- und Gesellschafts-Biol.* **13**.

(24) —— (1924). "Ueber einige Versuche, bei Säugetieren das Zahlenverhältnis der Geschlechter zu beeinflussen." *Arch. f. Rassen- und Gesellschafts-Biol.* **16**.

(25) Bodio (1895). "Movimento della Populazione." *Confronti Internazionali.*

(26) Bolaffio (1922). "Contributo al problema della determinazione del sesso." *Riv. Biol.* **4**.

(27) Bonnier (1923). "On alleged seasonal variations of the sex-ratio in man." *Zeits. f. ind. Abstammungs- und Vererbungslehre*, **32**.

(28) Boudin (1862). "De l'influence de l'âge relatif des parents sur le sexe des enfants." *Bull. Soc. d'anthrop. de Paris*, **2**.

(29) Brewster (1906). "Note on determination of sex in man." *Am. Anthrop.* **8**.

(30) Bucura (1905). "Geschlechtsverhältnis der Neugeborenen mit besonderer Berucksichtigung der mazerirten Kinder." *Zentralbl. f. Gynäk.* **29**.

(31) Bugnion (1910). "Les cellules sexuelles et la détermination du sexe." *Bull. Soc. Vaud. Sci. Nat.* **46**.

(32) Burton (1861). *The City of Saints and across the Rocky Mountains to California.* London.

(33) Campbell (1870). "On polygamy—its influence in determining the sex of our race and its effects on the growth of population." *Journ. Anthrop.* **8**.

(34) Carr (1867). *The History of the Rise and Progress of the Killerby, Studley and Warlaby Herd of Shorthorns.* London.

(35) Carvallo (1912). "La Masculinité dans les naissances humaines." *Comptes rendus Ass. franç. pour l'avancement des Sciences*, **41**.

(36) Castle (1910). "Russo on sex-determination and artificial modification of Mendelian ratios." *Am. Nat.* **44**.

(37) Cole and Davis (1914). "The effect of alcohol on the male germ cells studied by means of double matings." *Science*, N. S. **39**.

(38) Cooley and Slonaker (1925). "The effects of early and late breeding on the mother and the sex-ratio in the albino rat." *Am. Journ. Phys.* **72**.

(39) Copeman (1919). "Sex determination." *Proc. Zool. Soc.*

(40) Copeman and Parsons (1904). "Observations on the sex of mice." *Proc. Roy. Soc.* B, **73**.

(41) Corner (1923). "The problem of embryonic pathology of mammals, with observations upon intra-uterine mortality in the pig." *Am. Journ. Anat.* **31**.

(42) Crew (1925). "Prenatal death in the pig and its effect upon the sex-ratio." *Proc. Roy. Soc. Edin.* **46**.

(43) Cuénot (1899). "Sur la Détermination du Sexe chez les Animaux." *Bull. Sci. de France et Belg.* **32**.

(44) Danforth (1926). "Alcohol and the sex-ratio in mice." *Proc. Soc. Exp. Biol. and Med.* **23**.

(45) DARWIN (1871). *Descent of Man.* London.

(46) DAVENPORT (1920). "Influence of the male on the production of twins." *Medical Record,* **97.**

(47) —— (1920). "Influence of the male on the production of human twins." *Am. Nat.* **54.**

(48) DAVIES (1910). *Maternity.*

(49) DAVIS (1918). "Birth statistics for the birth registration area of the United States, 1916." *Bureau of Census.* Washington.

(50) —— (1919). Ditto, for 1917.

(51) —— (1920). Ditto, for 1918.

(52) DAWSON (1921). *The Causation of Sex.* London.

(53) DOHRN (1888). "Hat das enge Becken Einfluss auf die Entstehung des Geschlechtes?" *Zeits. f. Geburts. und Gynäk.* **14.**

(54) DONCASTER and MARSHALL (1910). "Effect of one-sided ovariotomy on the sex of offspring." *Journ. Gen.* **1.**

(55) DÜHRESSEN. Quoted by Hirsch (93).

(56) DUMONT (1894). "Natalité et Masculinité." *Revue Scientifique,* **1.**

(57) DUNCKER (1915). "Die Frequenzverteilung der Geschlechts-Kombinationen bei Mehrlinggeburten des Menschen und des Schweins." *Biol. Centralbl.* **35.**

(58) DÜSING (1884). "Die Regulierung des Geschlechtsverhältnisses bei der Vermehrung, etc." *Jenaische Zeits.* **17.**

(59) —— (1887, 1888, 1892). "Die Regulierung des Geschlechtsverhältnisses bei Pferden." *Landw. Jahrbücher,* **16, 17, 21.**

(60) DUTTON (1910). "The greater frequency of still-births and deaths under a year among males than females." *The Medical Press and Circular,* **89,** N. S.

(61) EAST and JONES (1919). *Inbreeding and Outbreeding.* Philadelphia.

(62) ELLIS (1904). *Man and Woman.* London.

(63) EWART (1911). "Sex-relationship." *Nature,* **85.**

(64) —— (1922). Letter in *Brit. Med. Journ.,* Feb.

(65) FRANCKE. Quoted by Newcomb (145).

(66) FRANZ (1898). "Zur Lehre des Aborts." *Beiträge zur Geburts.* **37.**

(67) FREEBORN (1916). "Determination of sex." *Can. Pract. Rev.* **41.**

(68) FÜRST (1886). "Knabenuberschuss nach Conception zur Zeit der postmenstruellen Anemie." *Arch. f. Gyn.* **18.**

(69) GEDDES and THOMSON (1889). *Evolution of Sex.* London.

(70) GINI (1908). *Il Sesso dal Punto di Vista statistico.* Milan.

(71) GOEHLERT (1882). "Ueber die Vererbung der Haarfarben bei den Pferden." *Zeits. f. Ethnol.* **14.**

(72) —— (1888). "Die Schwankungen der Geburtszahl nach Monaten." *Biol. Centralbl.* **8.**

(73) GÖRTZ (1852-4). *Reise um die Welt in den Jahren 1844-7.* Stuttgart. 3 vols.

(74) GRASSL (1912). "Einiges uber den Generationswechsel." *Arch. f. Rassen- und Gesellschafts-Biol.* **9.**

(75) GUAITA (1898). "Versuche mit Kreuzungen von verschiedenen Rassen der Hausmaus." *Ber. Naturforsch. Gesellsch. zu Freiburg,* **10.**

(76) GUIARD (1903). *Revue critique sur les lois de la formation des Sexes.* Paris.

(77) GÜNTHER (1923). "Letaldisposition und Sexualdisposition." *Naturwissenschaftliche Korrespondenz,* **1.**

(78) —— (1925). "Die Sexualproportion als Ausdruck einer Bionomie höherer Ordnung." *Zeits. f. Sexualwiss.* **12.**

(79) HAMMOND (1914). "On some factors controlling fertility in domestic animals." *Journ. Agric. Sci.* **6.**

(80) —— (1921). "Factors controlling fertility and foetal atrophy." *Journ. Agric. Sci.* **11.**

(81) HAMPE. Quoted by Düsing (58).

(82) HANSON and SHOLES (1924). "Seasonal differences in sex-ratio, litter size and birth weight of the albino rat under laboratory conditions." *Genetics,* **9.**

(83) HEAPE (1899). "Abortion, barrenness, and fertility in sheep." *Journ. Roy. Agric. Soc.* **10.**

(84) —— (1906). *The Breeding Industry.* Cambridge.

(85) —— (1908). "Note on Russo's attempt to show differentiation of sex in the ova of the rabbit." *Proc. Camb. Phil. Soc.* **14.**

(86) —— (1908). "Note on the proportions of the sexes in dogs." *Proc. Camb. Phil. Soc.* **14.**

(87) —— (1909). "The proportion of the sexes produced by white and coloured peoples in Cuba." *Phil. Trans.* B, **200.**

(88) HECKER (1874). "Ueber die Geburten älterer Erstgebärenden." *Arch. f. Gyn.* **7.**

(89) HENKE (1786). *Völlig entdecktes Geheimnis der Natur, etc.* Braunschweig.

(90) HENNEBERG (1897). "Wodurch wird das Geschlechtsverhältnis beim Menschen und den höheren Tieren beeinflusset?" *Anat. Hefte,* **7.**

(91) HERON (1906). "On the inheritance of the sex-ratio." *Biometrika,* **5.**

(92) Hertwig (1912). "Ueber den derzeitigen Stand des Sexualitätsproblems, nebst eigenen Untersuchungen." *Biol. Centralbl.* **32**.

(93) Hirsch (1913). "Ueber das Verhältnis der Geschlechter." *Zentralbl. f. Gynäk.* **37**.

(94) Hoffacker (1828). *Ueber die Eigenschaften welche sich bei Menschen und Thieren auf die Nachkommen vererben.* Tübingen.

(95) —— (1829). "Statistique médicale." *Ann. Publ. d'hygiène*, **1**.

(96) Hoffmann (1887). *Hat das enge Becken der Mutter Einfluss auf das Geschlecht des Fötus?* Würzburg.

(97) Huber (1915). "The development of the albino rat (*Mus norvegicus albinus*). 2. Abnormal ova, 1st–9th day." *Journ. Morph.* **26**.

(98) Huth (1887). *The Marriage of Near Kin.* London.

(99) Huxley (1922). "A statistical method of testing the biological causes underlying the excess of male births due to the war." *Eug. Rev.* **13**.

(100) —— (1924). "Sex determination and related problems." *Med. Sci. Abs. and Rev.* **10**.

(101) Jackson (1912). "On the recognition of sex through external characteristics in the young rat." *Biol. Bull.* **33**.

(102) Jastrzebski (1919). "The sex-ratio at birth." *Eug. Rev.* **11**.

(103) Jendrassik (1911). "Ueber die Frage des Knabengeburtenüberschuss und über andere Hereditätsprobleme." *Deutsche mediz. Wochenschr.* **37**.

(104) Jewell (1921). "Sex-ratios in foetal cattle." *Biol. Bull.* **41**.

(105) King (1911). "The effects of semi-spaying and semi-castration on the sex-ratio of the albino rat (*Mus norvegicus albinus*)." *Journ. Exp. Zool.* **10**.

(106) —— (1911). "The sex-ratio of hybrid rats." *Biol. Bull.* **21**.

(107) —— (1918). "Studies on inbreeding. 3. The effects of inbreeding with selection on the sex-ratio of the albino rat." *Journ. Exp. Zool.* **27**.

(108) —— (1921). "Birth mortality in the rat and in man." *Anat. Rec.* **20**.

(109) —— (1924). "Litter production and the sex-ratio in various strains of rats." *Anat. Rec.* **27**.

(110) King and Stotsenburg (1915). "On the normal sex-ratio and the size of the litter in the albino rat (*Mus norvegicus albinus*)." *Anat. Rec.* **9**.

(111) Knöpfel (1907). "Ueber die spezifische Sterblichkeit der beiden Geschlechter." *Allgem. statist. Archiv*, **7**.

(112) Kohl (1859). "Bemerkungen über die Bekehrung canadischer Indianer zum Christenthum und einige Bekehrungsgeschichten." *Das Ausland*, **32**.

(113) Kollmann (1890). "Der Einfluss des Alters der Eltern auf das Geschlecht der Geborenen nach statistischen Ermittelungen." *Allgem. Statist. Archiv*, Jahrg. 1890–1.

(114) Körösy (1898). *Die Sterblichkeit der Hauptstadt Budapest.* Berlin.

(115) Kroon (1917). "Jets over de verhouding der sterfte van mannen en vrouwen." *Nederlandsch Tijdschrift voor geneeskunde*, **61**. Jaargang 1.

(116) Kuntz (1920). "Retention of dead foetuses *in utero* and its bearing on the problems of superfoetation." *Anat. Rec.* **18**.

(117) Kuschakewitsch (1910). *Die Entwicklungsgeschichte der Keimdrüsen von Rana esculenta.* Festschr. R. Hertwig, **2**.

(118) Ladame (1904). *Contribution à l'étude de la mortinatalité suisse.* Bern.

(119) Lehr (1889). "Zur Frage der Wahrscheinlichkeit von weiblichen Geburten und Totgeburten." *Zeitschr. f. d. ges. Staatswissensch.* **45**.

(120) Lenhossek (1903). *Das Problem der geschlechtsbestimmenden Ursachen.* Jena.

(121) Lewis (1906). *Natality and Fecundity.* London.

(122) Lillie (1916). "The theory of the free-martin." *Science*, N. S. **43**.

(123) —— (1917). "The free-martin: the action of hormones in foetal life." *Journ. Exp. Zool.* **23**.

(124) Linden (1884). *Hat das enge Becken einen Einfluss auf die Entstehung des Geschlechtes?* Diss. Marburg.

(125) Little (1919). "Some factors influencing the human sex-ratio." *Proc. Soc. Exp. Biol. Med.* **16**.

(126) —— (1920). "Note on the human sex-ratio." *Proc. Nat. Acad. Sci. U.S.* **6**.

(127) Long, J. A. and Evans (1922). *The Oestrous Cycle in the Rat and Associated phenomena.* Mem. Univ. Calif. No. 6.

(128) Long, C. N. H. and Parkes (1924). "On the nature of foetal reabsorption." *Biochem. Journ.* **18**.

(129) MacDowell, Carleton and Lord (1925). "Data on the primary sex-ratio in the mouse." *Anat. Rec.* **31**.

(130) MacDowell and Lord (1925). "The sex-ratio in litters of mice classified by the total amount of pre-natal mortality." *Proc. Soc. Exp. Biol. Med.* **22**.

(131) —— (1926). "The relative viability of male and female mouse embryos." *Am. Journ. Anat.* **37**.

(132) —— (1926). "The sex-ratio of mice from alcoholised fathers." *Proc. Soc. Exp. Biol. Med.* **23**.

(133) MACHENS (1915). "Fruchtbarkeit und Geschlechtsverhältnis beim veredelten Landschwein." *Berliner Tierärztliche Wochenschrift*, **31**.

(134) LE MAIRE (1906). "Geschlechtsverhältnis der Neugeborenen mit besonderer Berücksichtigung der mazerirten Kinder." *Zentralbl. f. Gynäk.* **30**.

(135) MALCOLM (1924). "Sex-ratio in African peoples." *Am. Anthrop.* **26**.

(136) MALINS (1903). "Antenatal waste of life in nature and civilisation." *Journ. Obst. and Gyn. of British Empire*, **3**.

(137) MALL (1915). "On the fate of the embryo in tubal pregnancy." *Contributions to Embryology*, **1**, No. 1. Washington.

(138) MARMISSE (1867). *Recherches statistiques et comparées sur les mort-nés de la ville de Bordeaux*. Paris

(139) MAUREL (1903). "Étude sur la masculinité." *Revue Scientifique*. Paris.

(140) METHORST (1923). "La Prédominance des naissances masculines." *Metron*. **3**.

(141) MEYER (1917). "Intra-uterine absorption of ova." *Anat. Rec.* **12**.

(142) —— (1919). "Uterine, tubal and ovarian lysis and re-absorption of conceptuses." *Biol. Bull.* **36**.

(143) MILLER (1911). "Reproduction in the brown rat (*Mus norvegicus*)." *Am. Nat.* **45**.

(144) MÜLLER (1847). "Abhandlung uber den Bau der Molen, der medicinischen Facultät an der Universität Würzburg bei der Habilitation als Privatdocent vorgelegt." *Bonitas-Bauer*, Würzburg.

(145) NEWCOMB (1904). "A statistical inquiry into the probability of causes of the production of sex in human offspring." *Carnegie Institution Publications*, Washington.

(146) NICHOLS (1905). "Sex-composition of human families." *Am. Anthrop.* **7**.

(147) —— (1907). "The numerical proportions of the sexes at birth." *Mem. Am. Anthrop. Assoc.* **1**.

(148) D'ORANOVSKAIA (1900). *L'art de déterminer le sexe à volonté*. Paris.

(149) ORSCHANSKY (1894). "Étude sur l'hérédité normale et morbide." *Mémoires de l'Académie Imp. des Sci. de St Pétersbourg*, **42**.

(150) PARKER (1914). "Sex determination." *Science*, **39**.

(151) PARKER and BULLARD (1913). "On the size of litters and the number of nipples in swine." *Proc. Am. Acad. Arts and Sci.* **49**.

(152) PARKES (1921). "Sex heredity." *Sci. Pro.* **15**.

(153) —— (1923). "The respective sex-ratios of white and coloured races." *Man*, **23**.

(154) —— (1923). "Head length dimorphism of mammalian spermatozoa." *Q.J.M.S.* **67**.

(155) —— (1923). "Studies on the sex-ratio and related phenomena. 3. Influence of size of litter." *Ann. App. Biol.* **10**.

(156) —— (1923). "Studies on the sex-ratio and related phenomena. 4. The frequencies of sex combinations in pig litters." *Biometrika*, **15**.

(157) —— (1924). "Studies on the sex-ratio and related phenomena. 1. Foetal retrogression in mice." *Proc. Roy. Soc.* B, **95**.

(158) —— (1924). "Studies on the sex-ratio and related phenomena. 2. The influence of the age of the mother on the sex-ratio in man." *Journ. Gen.* **14**.

(159) —— (1924). "Studies on the sex-ratio and related phenomena. 5. The sex-ratio in mice and its variations." *Brit. Journ. Exp. Biol.* **1**.

(160) —— (1924). "The factors governing the mammalian sex-ratio." *Sci. Prog.* **18**.

(161) —— (1924). "The frequencies of sex combinations in human families." *Eugenics Review*, **16**.

(162) —— (1924). "Some aspects of reproduction considered in relation to eugenics." *Eugenics Review*, **15**.

(163) —— (1924). "Fertility in mice." *Brit. Journ. Exp. Biol.* **2**.

(164) —— (1925). "Studies on the sex-ratio and related phenomena. 6. The effect of polygyny." *Ann. App. Biol.* **12**.

(165) —— (1925). "Studies on the sex-ratio and related phenomena. 7. The foetal sex-ratio in the pig." *Journ. Agric. Sci.* **15**.

(166) —— (1926). "Studies on the sex-ratio and related phenomena. 8. The seasonal sex-ratio in the pig." *Zeits. fur Ind. Abs. u. Verer.* **40**.

(167) —— (1926). "Studies on the sex-ratio and related phenomena. 9. Further observations on sex-ratio and fertility in mice, 1922-5." *Brit. Journ. Exp. Biol.* **4**.

(168) —— (1925). "The effects on fertility and the sex-ratio of sub-sterility exposures to X-rays." *Proc. Roy. Soc.* B, **98**.

(169) —— (1926). "The physiological factors governing the proportions of the sexes in man." *Eugenics Review*, **17**.

(170) PARKES and DRUMMOND (1925). "The effect of vitamin B deficiency on reproduction." *Proc. Roy. Soc.* B. **98**.

(171) —— (1926). "The effects of fat soluble vitamin deficiency on reproduction in the rat." *Brit. Journ. Exp. Biol.* **3**.

(172) Pearl (1906). "On the mean duration of life of individuals dying within a year after birth." *Biometrika*, **4**.

(173) —— (1917). "The control of the sex-ratio." *Maine Agric. Exp. Stat. Bull.* No. 261, Pt 3.

(174) —— (1917). "The experimental modification of germ cells." *Journ. Exp. Zool.* **22**.

(175) —— Quoted by Wentworth (240).

(176) Pearl, M. and R. (1908). "On the relation of race-crossing to the sex-ratio." *Biol. Bull.* **15**.

(177) Pearl and Parshley (1913). "Data on sex determination in cattle." *Biol. Bull.* **24**.

(178) Pearl and Salaman (1913). "The relative time of the fertilisation of the ovum and the sex-ratio amongst Jews." *Am. Anthrop.* **15**.

(179) Pearson (1908). *The Chances of Death and other Studies in Evolution.* London.

(180) Pike (1907). "A critical and statistical study of the determination of sex, particularly in human offspring." *Am. Nat.* **41**.

(181) Pinard and Magnan (1913). "Sur la fragilité du sexe mâle." *Comptes rendus hebdomadaires des séances de l'Académie des Sciences*, **156**.

(182) Pitt-Rivers (1924). "Variations in sex-ratios as indices of racial decline." *Man*, **24**.

(183) Ploss (1858). "Ueber die das Geschlechtsverhältniss der Kinder bedingenden Ursachen." *Monatsch. f. Geburtskunde u. Frauenkunde*, **12**.

(184) —— (1861). "Ein Blick auf die neuesten Beiträge zur Frage über das Sexualverhältnis der Neugeborenen." *Monatsch. f. Geburtskunde u. Frauenkunde*, **17**.

(185) Powers (1877). *Tribes of California.* Washington.

(186) Priestley. Quoted by Routh (201).

(187) Prevost (1829). "Del efecto de la Legitimidad sobre la Proporcion de los Nacimientos de Diversos Senos." *Biblioteca Universal*.

(188) Prinzing (1906). *Handbuch der medizinischen Statistik.* Jena.

(189) —— (1907). "Die Ursachen der Totgeburt." *Allgem. Statist. Archiv*, **7**.

(190) Pryll (1916). "Kohabitationstermin und Kindsgeschlecht." *Münch. med. Wochenschr.* **63**.

(191) Punnett (1904). "On nutrition and sex determination in man." *Proc. Camb. Phil. Soc.* **12**.

(192) —— (1909). "On the alleged influence of lecithin upon sex determination in rabbits." *Proc. Camb. Phil. Soc.* **15**.

(193) Quételet. Quoted by Bugnion (31).

(194) Rauber (1900). *Der Ueberschuss an Knabengeburten und seine biologische Bedeutung.* Leipzig.

(195) Rawlings (1922). "The interstitial gland and the sex problems." *Brit. Med. Journ.* July 1st.

(196) Registrar-General (1919). *Report.*

(197) Riddle (1916). "Success in controlling sex." *Journ. Hered.* **7**.

(198) Robertson (1917). "A mule and a horse as twins, and the inheritance of twinning." *Univ. Kansas Sci. Bull.* **10**.

(199) Robinson (1921). "Prenatal death." *Edin. Med. Journ.* **26**.

(200) Rosenfeld (1900). "Die Sexualproportion in Oesterreich in den Jahren 1895 6." *Wien. med. Bl.* **23**.

(201) Routh (1914). "Ante-natal hygiene." *Brit. Med. Journ.* Feb. 14th.

(202) Russell (1891). "Breeding statistics." *Maine Agric. Exp. Stat. Ann. Rep.*

(203) Russo (1907). "Modificazione sperimentale dell' elemento epiteliale dell' ovaia dei mammiferi." *Atti (Rend.) R. Accad. Lincei*, **16**.

(204) —— (1907). "Metodi adoperati per aumentare artificialmente la produzione del sesso femminile nei conigli e per fissare nella prima generazione degl' incroci le varietà recenti." *Atti (Rend.) R. Accad. Lincei*, **16**.

(205) —— (1908). "Sulla origine e sulla funzione dell' apparato mitocondriale nelle cellule sessuali dei mammiferi." *Boll. Accad. Gioenia Sci. Nat. Catania.*

(206) —— (1909). *Studien über die Bestimmung des weiblichen Geschlechtes.* Jena.

(207) Rust (1902). *Das Geschlecht der Fehl- und Totgeburten.* Strassburg.

(208) Sadler (1830). *The Law of Population.* London.

(209) St Hilaire (1824). "Note sur un fait remarquable pour la théorie de la procréation des sexes." *Ann. Sc. Nat.* **1**.

(210) Sanderson (1879). "Polygamous marriage among the Kaffirs of Natal and countries around." *Journ. Anthrop. Inst.* **8**.

(211) Savorgnan (1921). "L'aumento delle nascite maschili durante la guerra." *Metron.* **1**.

(212) Schultz (1918). "Studies in the sex-ratio in man." *Biol. Bull.* **34**.

(213) —— (1921). "Sex incidence in abortions." *Contributions to Embryology*, **12**.

(214) Schultze (1903). "Zur Frage von den geschlechtsbildenden Ursachen." *Arch. f. Mikr. Anat.* **63**.

(215) Seligson (1895). "Zur Bestimmung und Entstehung des Geschlechts." *Centralbl. f. Gynäk.* **19**.

(216) Siegel (1916). "Zur willkürlichen Geschlechtbestimmung." *Münch. med. Wochenschr.* **63**.

(217) —— (1921). "Beiträge zur menschlichen Schwangerschaftsdauer." *Zentralbl. f. Gynäk.* **45**.

(218) SLONAKER and CARD (1923). "The effect of a restricted diet. V. On mortality, cannabalism, and the sex-ratio." *Am. Journ. Phys.* **64**.

(219) SORMANI (1883). "L'influence des Saisons en Italie sur la distribution des sexes dans les naissances et dans les décès." *Quatr. Cong. internat. d'Hyg. et de Démog.* **2**.

(220) SPECHT (1916). "Ueber das Geburt bei Minderjährigen." *Zentralbl. f. Gynäk.* **40**.

(221) STADLER (1878). "Ueber den Einfluss des Alters der Mutter auf das Geschlecht des Kindes." *Med. chir. Centralbl. Wien*, **13**.

(222) STARKWEATHER (1883). *The Law of Sex.* London.

(223) STEWART (1910). "The sex and age incidence of mortality from pulmonary tuberculosis in Scotland and in its groups of registration districts since 1861." *Proc. Roy. Soc. Edin.* **31**.

(224) STIEDA (1875). "Das Sexualverhältnis der Geborenen." *Stat. Mittheil.* **5**.

(225) STOCKARD and PAPANICOLAOU (1916). "A further analysis of the hereditary transmission of degeneracy." *Am. Nat.* **50**.

(226) STRAHL and HENNEBERG (1902). "Ueber Ruckbildungserscheinungen am graviden Säugtier-uterus." *Anat. Anz.* **20, 21**.

(227) SUMNER, McDANIEL and HUESTIS (1922). "A study of influences which may affect the sex-ratio of the deer mouse (*Peromyscus*)." *Biol. Bull.* **43**.

(228) TAUSSIG (1910). *Prevention and Treatment of Abortion.* St Louis.

(229) TERRY (1917). *The Secret of Sex.* New York.

(230) THOMAS (1913). *Report on the Ibo-speaking peoples of Nigeria*, Pt 1. London.

(231) —— (1923). "Sex-ratio and race." *Man*, **23**.

(232) THURY (1863). *Ueber das Gesetz der Erzeugung der Geschlechter.* Leipzig.

(233) —— (1863). *Mémoire sur la loi de production des Sexes.* Genève: Cherbuliez.

(234) —— (1864). "Loi de la production des sexes." *Revue de Thérap. Méd.-Chir.*

(235) TREICHLER (1895). "Statistische Untersuchungen uber den Einfluss des Altersverhältnisses der Eltern und der Geburtenfolge auf die Haufigkeit der Totgeburten im Kanton Zürich." *Zeit. f. schweiz. Stat.* **31**.

(236) TSCHUPROV (1916). "Zur Frage des sinkenden Knabenuberschusses unter den eheliche Geborenen." *Bull. Inst. Int. de Stat.* **20**.

(237) VENN (1888). *The Logic of Chance.* London.

(238) WAITZ (1859). *Anthropologie der Naturvolker.* Leipzig.

(239) WELDON (1906). "On heredity in mice from the records of the late W. F. R. Weldon. Pt 1. On the inheritance of the sex-ratio and the size of litter." *Biometrika*, **5**.

(240) WENTWORTH (1914). "Sex in multiple births." *Science*, N.S. **39**.

(241) WESTERMARCK (1921). *History of Human Marriage.* 5th ed. London.

(242) WHITEHEAD. Quoted by Routh (201).

(243) WILCKENS (1886). "Untersuchungen uber das Geschlechtsverhältnis und die Ursachen der Geschlechtsbildung in Haustieren." *Biol. Centralb.* **6**.

(244) WILLIAMS (1912). *Obstetrics.* New York.

(245) WINCKEL (1903). *Handbuch der Geburtshilfe.* Wiesbaden.

(246) WODSEDALEK (1914). "Spermatogenesis in horse." *Biol. Bull.* **27**.

(247) —— (1920). "Studies on the cells of cattle with special reference to spermatogenesis, oogonia and sex-determination." *Biol. Bull.* **38**.

(248) WOODS (1906). "The non-inheritance of sex in man." *Biometrika*, **5**.

(249) YERKES (1907). *The Dancing Mouse.* New York.

(250) ZELFNY and FAUSI (1915). "Size dimorphism in spermatozoa." *Journ. Exp. Zool.* **18**.

(251) —— (1901). "Statistik der Eingeborenen-Bevolkerung der Neu-Lanenburg-Gruppe." *Mittheil. deutsch. Schutzgeb.* **14**.

ON THE PRESENT POSITION OF THE MITO-CHONDRIA AND THE GOLGI APPARATUS

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INTRODUCTION.

HAPPILY the period of scepticism even about the *existence of* such formed elements in the cell as the mitochondria and the Golgi apparatus is long over, since it has been abundantly made clear that the mitochondria and, in a number of cases recently demonstrated by Parat and Gatenby, even the Golgi apparatus are visible in the living cell. It has been shown that the existence of the mitochondria and the Golgi apparatus is all but universal. We have now passed the descriptive stage and attention is being increasingly focussed on the functions of these bodies in different kinds of cells. The cell, however, is a complex functioning unit and its function is the sum total of a long series of physico-chemical processes yet little understood and a slight variation in the behaviour of one cell-component may change the behaviour of the others; hence we find that the behaviour of the mitochondria and the Golgi apparatus may be different even in closely allied forms, and what may be true of one cell may not be true of another.

Unfortunately most workers on the cell have confined their attention either to the nucleus or to the cytoplasm, with the result that some have ascribed the principal rôle to the nucleus in histogenesis and others to the mitochondria and the Golgi apparatus. The importance of the study of a cell from both the nuclear and the cytoplasmic points of view cannot be over-emphasised.

An attempt has been made in this review to discuss the mitochondria and the Golgi apparatus from as many aspects as possible except the changes which they undergo under pathological conditions. The reactions of the mitochondria and the Golgi apparatus to various pathological conditions are not in the least specific, so that no useful purpose will be served by discussing them here. Reference may, however, be made to Cowdry (1924) for these phenomena.

GOLGI APPARATUS.

Technique.

In 1898 the Italian neurologist, Golgi, discovered an internal reticular apparatus (apparato reticolare interno) in nerve cells after treatment with his bichromate and nitrate of silver method (rapid process, see Lee, 1921). Since then various methods (Veratti, Golgi, Ramón y Cajal, Da Fano, Sjövall, Kopsch and Mann Kopsch, see Lee, 1921) have been devised for the demonstration of the Golgi apparatus in almost all kinds of cells. All these methods consist in fixation by reagents containing no acetic acid or other lipoid-solvent and subsequent impregnation by metallic silver or osmium. For various modifications of the osmic acid methods reference may be made to Ludford (1924 and 1925 *a*) and to Nassonov (1923), who describe in detail Kolatchev's osmium technique which is now recognised to be the most efficient method for the demonstration of the Golgi apparatus. Flemming-without-acetic, Altman's, Benda's, Bensley-Cowdry's, Champy-Kull's or Regaud's methods (see Lee, 1921) may also demonstrate the Golgi apparatus in somatic and male germ cells, but in the female germ cells it seldom appears with these methods. All these methods, especially the silver and the osmium methods, require considerable patience and experimentation and the beginner should not feel disappointed if his first preparations are not successful. Great care should also be exercised in interpreting the preparations, as a slight defect in the technique is often a fruitful source of error particularly with regard to the form and size of the apparatus.

According to Cowdry (1924, p. 334), "methods have not yet been devised by which the Golgi apparatus may be studied in the living cells of Vertebrates." This clearly shows that the apparatus has a low refractive index, even lower than that of the mitochondria which can be studied in the living cell. Further, Cowdry (1924, p. 340) says: "Nor has it been possible to stain it with vital dyes, although many have been tried." Parat and his collaborators have, however, subsequently been able to stain the Golgi apparatus (which, according to them, consists of vacuoles collectively called the "vacuome") with the basic vital dye, neutral red, in the cells of the salivary glands of the larva of *Chironomus* (*Comp. rend. des séances de l'Acad. des sci.* Sept. 15, 1924), in the male genital cells of *Helix* (*Comp. rend. des séances de la Soc. de biol.* March 20, 1926), in the nerve cells of *Hirudo medicinalis* and the larva of *Paracentrotus*, and in both the genital and the somatic cells of Vertebrates (*Comp. rend. des séances de l'Acad. des sci.* Oct. 27, 1924 and March 22, 1926, and *Comp. rend. des séances de la Soc. de biol.* March 20, 1926 and Feb. 26, 1926). Parat seems to be the only worker who has made an extensive study of the Golgi apparatus with vital dyes and his views, therefore, must claim special attention. The study of the "vacuome" in the salivary glands of *Chironomus* is remarkably facilitated by neutral red. The larvae are placed in water containing the dye, and later the salivary glands are removed and studied. Neutral red diffuses first into the protoplasm; then it goes into numerous small vacuoles

uniformly distributed in the cell. In these vacuoles the colouring matter is precipitated in small crystals. The vacuoles differ in size, the largest being near the nucleus. Those near the nucleus are filled with red crystals of the dye and have the appearance of mulberries. Neutral red has no effect on the mitochondria for the study of which Janus green, violet dahlia, and Bismarck brown are used.

Rau, Brambell and Gatenby (1925) have also shown by a series of convincing photographs that "the apparatus can easily be observed in the living unstained spermatocytes of *Helix*, and can be stained either with Janus green or by the same method followed by rapid treatment with iodine vapour according to the Lewis method. It can also be observed in fresh spermatocytes of *Helix* treated for a few seconds with osmic vapour from 2 per cent. solution. Its demonstration in fresh cells is thus on a par with that of the mitochondria." Indeed, one photograph of a living cell stained with Janus green which these writers give shows distinctly individual dictyosomes surrounding the central "sphere substance."

Parat and Gatenby, therefore, have proved that the Golgi elements, both in the Invertebrates and the Vertebrates, are denser than the surrounding cytoplasm. In a recent paper, Bowen (*Quart. Journ. Micr. Sci.* April 1926) finds discrete Golgi elements in the lumina of the oil glands of duck. These elements are liberated into the lumina when the whole cell is disrupted. Bowen, therefore, says, "that the Golgi apparatus has in some cases a composition sufficiently sturdy to maintain itself in the absence of its usual environment, can no longer be questioned. I am inclined to agree with Gatenby and his co-workers that some authors have gone too far in their emphasis on the instability or lack of actual material differentiation of the Golgi apparatus." Kite and Chambers (Cowdry, 1924, p. 340), on the other hand, in their micro-dissection studies on the vertebrate cells, did not encounter any "areas of resistance suggestive of the presence of a rigid network" and further movements were produced in the cytoplasm when the cells were carefully crushed (Cowdry, 1924, p. 340). These observations of Kite and Chambers do not contradict the view that the Golgi apparatus is a definite and permanent cell-component, because Parat has made it abundantly clear (as will appear later) that the so-called Golgi network of the Vertebrate somatic cells is an artifact produced by the precipitation of metallic silver and osmium in the interior, at the periphery or between the vacuoles that constitute the Golgi apparatus.

Form.

"Usually in the somatic cells of Mammals and the majority of Vertebrates, the Golgi apparatus is seen in the form of a more or less dense network consisting of anastomosing strands of material of uneven girth but of smooth contours" (Cowdry, 1924, p. 334). Bowen (1926), however, emphasises the fact "that in the mucous cells of the submaxillary gland (cat) the Golgi apparatus is not a network of simple thread-like strands but (except perhaps in the latest stages) could be more appropriately compared with an irregular and much perforated platework, the perforated condition leading to the appearance of a very complicated reticulum."

In the germ cells of Vertebrates and both in the somatic and germ cells of Invertebrates, however, the apparatus consists of separate rods, crescents, rings or sometimes granules, either distributed at random throughout the cytoplasm or aggregated together into a more or less compact body. In the localised form the "dictyosomes" or the "batonettes" or the "Golgi bodies" surround a clear non-staining area, the "sphere substance," or the "idiosome," or the "idiozome," or the "archoplasm," or again, the "centrosphere," embedded in which are the centrioles. The word "archoplasm" is, however, unfortunate, as it may be confused with the cytoplasmic differentiation of the same name in connection with the mitotic figure with which it has no relation whatsoever. The American writers have adopted the words "sphere substance" or "idiosome." This topographic relation of the Golgi elements with the centrioles, now fully established, was observed by Heidenhain (1900), who described the Golgi elements as the "pseudochromosomes" in the spermatocytes of *Proteus* at a time when the Golgi apparatus was not yet even discovered in the germ cells. There are, however, some exceptions to this general rule. For example, in the ciliated epithelium, such as that of the epididymis of the Mammalia, the centrosome is associated with the cilia and the Golgi apparatus lies between the nucleus and the centrosome (Rau and Ludford, 1925). The mitochondria may also show grouping about the centrioles, particularly in the spermatogonia and young oocytes, but it is never so definite as in the case of the Golgi apparatus. My own experience shows (and some other workers have also observed) that on account of faulty technique, *e.g.* extreme reduction of silver by hydroquinone (Da Fano's method), the dictyosomes may join together and give the appearance of a network.

In the diffuse form of the Golgi apparatus the dictyosomes lie separately in the cytoplasm and in many cases it is possible to see the "sphere substance" attached to each dictyosome.

The localised type of the Golgi apparatus may pass into the diffuse form or *vice versa*. Cajal (1908, etc.) showed that even in the nerve cells of Vertebrates the Golgi network may break up into smaller networks or even into separate batonettes. Gatenby (1919) and Hirschler (1918), working independently on Pulmonates, have shown a Golgi apparatus of the localised type in the youngest oocyte, which, however, becomes diffuse with the growth of the oocyte and remains so as late as the gastrula stage. The work of Golgi, Sjövall, Marcora, etc. (see Hirschler, 1918) shows a localised type of Golgi apparatus in the later stages of development of birds and mammals; but Fañanas (1912; quoted by Wilson, p. 51) has shown a diffuse form of Golgi apparatus in the early stages of hen's eggs (44 hours and later). Recently Rau and Ludford (1925), working on the development of the neurones, have shown that in the spinal ganglia of the chick of four days the Golgi apparatus is in the form of a cluster of granules or rodlets, grouped round the centrosphere, at one side of the nucleus. In a seven-day chick the Golgi apparatus increases in size and begins to spread further round the nucleus. In the adult ganglia the apparatus is more or less scattered throughout the cell. In a number of eggs which the writer has studied, *e.g. Lithobius* (1924), *Buthus* (1925 *b*), *Julus* (in press),

Lumbricus (in press) and Spider (in press), the localised type of the Golgi apparatus of the young oocyte gives rise to the diffuse form when the oocyte grows. These facts point strongly towards the conclusion that the "sphere substance" of the localised type of Golgi apparatus is the sum total of the "spheres" of the individual dictyosomes that aggregate together. They also perhaps show (especially the case of Pulmonates) that the localised type is only a secondary condition. This view is further supported by the study of dictyokinesis in which the localised type of the apparatus may become diffuse and also by the re-aggregation of some of the diffuse Golgi elements to form the acroblast, two points to be discussed later.

The above facts and others, such as the consistency with which both the localised network-like Golgi apparatus of the vertebrate somatic cells and the batonette type of Golgi apparatus of the invertebrate somatic cells, as well as the germ cells of both the Vertebrates and the Invertebrates, are revealed by the various osmium and silver techniques, abundantly make it clear that they are strictly homologous structures. For further proof see Gatenby and Brambell (1923) and Gatenby, etc. (1925). It must, however, be remembered that the differentiation of a Golgi element into a chromophilic rim and a chromophobic area (sphere substance) so commonly met with in the somatic cells of Invertebrates, as well as in the germ cells of both Vertebrates and Invertebrates, is rarely met with in the network-like Golgi apparatus of the vertebrate somatic cells. Discrete Golgi elements like those of the invertebrate cells have also been found in the oil glands of the chick and the duck and in the so-called red portion of the Harderian glands of rabbit (Bowen, *Quart. Journ. Micr. Sci.* April 1926).

The recent remarkable researches of Parat, however, have revolutionised the whole idea about the form of the Golgi apparatus. According to this worker, who has used vital stains extensively, the Golgi apparatus consists of vacuoles which may appear like a network but which, in fixed cells, are an artifact produced by the precipitation of metallic silver and osmium. In many somatic cells of Vertebrates in which a Golgi network had been described, Parat has shown by the use of neutral red that such a network is more an artifact than the image of reality, *e.g.* in the glands of the stomodæum and the intestine of Batraciens and the pancreas of fish and Amphibians (*Comp. rend. des séances de la Soc. de biol.* Jan. 17, 1925 and *Comp. rend. des séances de l'Acad. des sci.* Oct. 27, 1924). To the writer the above view of Parat seems to be nearest the truth for the following reasons. Firstly, Parat has actually seen vacuoles in the living somatic cells of Vertebrates stained with neutral red in exactly the same position in the cell in which a network had been described by those who had worked with silver nitrate and osmium tetroxide; secondly, sharp black ring-like and crescent-shaped Golgi elements with their chromophobic "sphere substance" have been described not only in all kinds of cells of the Invertebrates but also in the genital cells of the Vertebrates. It is easy to understand how a vacuole treated with silver and osmium would appear like a ring or a crescent. The granular type of Golgi element would appear to result from the complete blackening of a very small vacuole; thirdly, Kite and Chambers in their microdissection studies on the vertebrate somatic cells did not encounter

any areas of resistance suggestive of a rigid network and further movements were actually produced in the cytoplasm when the cells were carefully crushed; fourthly, networks can be artificially produced by extreme reduction of silver and osmium, especially the former, in the cells of Invertebrates; fifthly, Cajal (1908, etc.) has shown that even in the nerve cells of Vertebrates the Golgi network may break up into separate batonettes; sixthly, in the eggs of spiders (Nath), *Scolopendra* (Nath and Hussain, unpublished) and the firefly *Luciola* (Nath and Melita, unpublished) the vacuole-like or the ring-like Golgi elements swell up and give rise to fatty yolk spheres which, on decolorisation in turpentine, appear as clear vacuoles giving a frothy appearance to the whole egg; and lastly, discrete Golgi elements like those of the Invertebrate cells have also been found in the oil glands of the chick and the duck and in the so-called red portion of the Harderian gland of rabbit (Bowen).

Chemical composition.

The chemical composition of the Golgi apparatus is supposed to be "much like that of the mitochondria, *i.e.* proteid in some way linked with lipin materials" (Gatenby, 1919) and the Golgi apparatus may possibly be composed of lecithalbumin (Weigle, 1912). This is, of course, only inferred from the solubility of the apparatus in acetic acid, alcohol, chloroform, ether and other lipoid-solvents. It is very likely that there is some proteid substance in the Golgi apparatus; for in many cases I have observed, *e.g.* the spermatocytes of *Lithobius forficatus* (1925 a), that the Golgi elements are not completely destroyed even in Gilson's fluid and that they remain as highly distorted bodies of irregular shape, described earlier as "formations ergastoplasmiques" by the Bouin brothers or as "metaplasm" by Blackman. Further, it seems likely that the exact relationship between the proteid and the lipin material is slightly different in different cells or even in the same cell at different times.

Parat (*Comp. rend. des séances de l'Acad. des sci.* April 6, 1925 and *Comp. rend. des séances de la Soc. de biol.* March 21, 1925), however, denies the lipoidal nature of the Golgi apparatus. He insists that osmium tetroxide is not a specific for lipoids but for fats. The content is mostly a liquid and its reaction is acidic. The absence of coagulum leads us to think that we have to deal in the majority of cases with the solution of crystalloids. But it is a fact that certain colloids not miscible with protoplasm can accumulate in the vacuoles like the aleurone grains in the vegetable cells. If the vacuome is not very much saturated with colloids it has a remarkable affinity for certain basic colours like neutral red.

Division.

It is premature to attack the problem as to whether the Golgi elements always arise by division from pre-existing ones or *de novo* in the cytoplasm. Gatenby believes that they have a marked degree of independence and can assimilate, grow and divide in the cytoplasm. That they can grow in the cytoplasm is proved by the writer's observations and also of others on a number of eggs in which the Golgi elements swell up. To prove that the Golgi elements are self-perpetuating two things

are necessary. Firstly, the Golgi apparatus or its representative should be present in every cell of Vertebrates and Invertebrates—a generalisation actually made by Gatenby (1919). Cowdry (1924), however, cites the non-nucleated red blood cells of mammals as an exception. Brambell and Bhattacharya (1925) describe a Golgi apparatus in the nucleated red blood corpuscle of Sauropsida and remark: "In the young, nucleated red blood cells of Mammals, the mitochondria are abundant, but these, along with the nucleus, disappear *pari passu* with the later differentiation of the cell (Cowdry). It must be remembered, however, that the fully differentiated red blood corpuscle is not, strictly speaking, a cell according to the old definition of Leydig and Schultze. It is therefore possible that the Golgi elements, if present in the younger stages of the mammalian red blood cells, may undergo a similar change." But unless a Golgi apparatus is actually discovered "in the younger stages of the mammalian red blood cells" the objection of Cowdry stands. Secondly, the actual division stages of the Golgi elements must be shown before we can consider these elements as self-perpetuating. Gatenby (1919) shows such division stages in the eggs of *Lymnoea*. He shows breaks in the chromophilic rim of the Golgi elements and interprets these as possible division stages. I have myself seen such appearances in the spermatocyte of *Lithobius*, but such appearances may possibly be due to the close aggregation of more than one batonette or simply to some weak points in the batonette. Similarly, Bowen (1920) has shown that in the Hemipteran spermatogenesis the longitudinal slit of the Golgi element so conspicuous in the prophase is an optical illusion produced by the presence of a non-staining axial substance. In a recent paper, Bowen (*Quart. Journ. Micr. Sci.* April 1926) has furnished unquestionable evidence that in the meibomian glands of the cat the Golgi network grows and fragments. This supports the much-criticised view of Gatenby that the Golgi elements have a marked degree of independence and can assimilate, grow and divide in the cytoplasm.

Holmgren's canalicular system.

Soon after the Golgi apparatus was discovered, Holmgren (1899 and later), and several other workers, found a system of clear, intracellular canals in the cytoplasm of several kinds of cells. These investigators regard the network-like Golgi apparatus as a system of intracellular canals (canalicular system) filled with some lipoid fluid which after treatment with silver or osmium appear as solid filaments. Holmgren regarded these canals as ingrowths from the neighbouring cells which become hollow and give rise to a system of intracellular canals, still retaining their connection with the exterior and having a nutritive function. Hence the terms "canalicular system," "trophospongium" and the "Golgi apparatus" were used as synonyms for the same structure. In fact Cajal (1908) proposed the name of "Golgi-Holmgren canals" to include both the structures.

Later researches, however, seem to have proved that the Golgi apparatus is a structure quite distinct from a system of fibrils formed as ingrowths from the neighbouring cells. Duesberg (1914, 1920) is "of the opinion that the two formations are identical in neurones and non-nervous cells which possess a localised

trophospongium (canalicular system), but that in non-nervous cells, with a diffuse trophospongium spread throughout the cytoplasmic area, they cannot be the same, because the Golgi apparatus, on the contrary, is restricted in distribution, being localised at one pole of the nucleus (except in lutein cells)." Penfield (1921), working on the spinal ganglion cells of a cat seventeen days after the section of the posterior nerve roots, demonstrated a Golgi apparatus, but when the same cells were bleached and stained with iron haematoxylin he observed a system of clear canals which did not in any way correspond with the remains of the Golgi apparatus. Hence he concluded that the Golgi apparatus and the " canalicular canals " are quite distinct structures.

On the other hand, Cajal (1908), Bensley (1910) and Cowdry (1924) agree with Holmgren that the Golgi network represents a system of lipoid-containing intracellular canals which after treatment with silver and osmium appear as solid filaments but they do not believe that the ingrowths are connected with the neighbouring cells. "Observations are not lacking that there is often a close correspondence between systems of clear canals and blackened networks in normal nerve cells. When preparations of the Golgi apparatus in acinus cells of the pancreas are bleached and re-stained with iron haematoxylin, clear canals are seen in place of the blackened networks " (Cowdry). The network-like Golgi apparatus of the vertebrate somatic cells may in some cases be identical with Holmgren's canalicular system but obviously it is impossible to regard the diffuse type of Golgi apparatus as a system of intracellular canals representing ingrowths from neighbouring cells, especially when we remember that in dictyokinesis the diffuse Golgi·elements may become oriented either towards the poles or the equator of the spindle. But the network-like Golgi apparatus itself has been shown by Parat to be an artifact caused by the precipitation of metallic silver and osmium in the interior, at the periphery or between the vacuoles that constitute the Golgi apparatus. In the words of Parat himself—" Il est néanmoins certain que la 'trophosponge' résulte d'une déformation considérable de l'appareil vacuolaire et qu'elle est bien plus un *artefact* que l'image de la réalité." (*Comp. rend. des séances de l'Acad. des sci.* Sept. 29, 1924.)

Distribution.

In the Metazoa the Golgi apparatus seems to be of almost universal occurrence. It is true that it has not yet been demonstrated in the non-nucleated mammalian red blood cells or in the desquamating epithelial cells, but such cases are instances of cells that are approaching senile decay and it seems likely that the Golgi material in such cells atrophies. Brambell and Bhattacharya have discovered a typical Golgi apparatus in the nucleated red blood corpuscles of Sauropsida and we might expect to find it in the young nucleated mammalian red blood corpuscles also.

Amongst the Protozoa a Golgi apparatus was described first by Hirschler (1914) and later in the Sporozoon *Adelea* by Gatenby and King (1923). Recently it has been described by M. Ph. Joyet-Lavergne (1924) in *Aggregata Eberthi* and *Adelina dimidiata*, confirming the account of Gatenby and King. Most recently King (1926) has described it in the Neosporidium, *Haplosporidium Chitonis*. In all these

Protozoan forms the Golgi apparatus is essentially of the same form as in the higher Invertebrates.

In plants the existence of a Golgi apparatus is still doubtful. Bensley (1910), however, has made an elaborate study of the "canalicular system" both in plants and animals. By the study of both living and fixed cells he discovered in the onion root tip a system of clear canals which with the growth of the cells gives rise to the familiar plant-cell vacuole and which is similar in many details to the "canalicular system" of Holmgren in the animal cell. He, therefore, suggests "that the network* of canals found in so many animal cells is the physiologic and morphologic equivalent of the vacuolar system in the plant cell." Guilliermond and Mangenot (1922), working on barley cells, also believe that the plant-cell vacuole is homologous with the "canalicular system" of Holmgren in animals. The researches of Parat who has shown that the Golgi apparatus in animal cells consists of a number of vacuoles lend strong support to the theory that the vacuolar system of plants is homologous with the Golgi apparatus of the animal cell.

A Golgi apparatus has not yet been discovered in the Ciliata, but the line of argument adopted above by Bensley that the plant-cell vacuole is the homologue of the Golgi apparatus in animal cells seems to have led Nassonov (1924) to homologise the contractile vacuole of the Ciliates with the Metazoan Golgi apparatus. Nassonov, by means of osmic acid, has been able to blacken the wall of the contractile vacuole and the associated drainage canals. "He claims that this osmiophile membrane has the property of secreting an osmotically active substance into the lumen of the contractile vacuole, and also itself has the distinctive character of a semipermeable membrane." "Furthermore, Nassonov gives several reasons for homologising the Golgi apparatus of the Metazoa with the contractile vacuole cortex of the Protozoa: first, both organellae are not visible *in vivo*; second, their morphology is similar, both being bladders with an osmiophile wall; third, the sponge (the Metazoan nearest to the colonial Protist) has a Golgi apparatus which looks like a contractile vacuole; and, finally, the Golgi apparatus of the Metazoan cell also has the power of secreting various substances" (*vide* Gatenby and King, 1925). These authors have given a criticism of Nassonov's view. They agree with Nassonov that the wall of the contractile vacuole can be blackened with osmium tetroxide, but those who are familiar with the use of this reagent know that too much reliance should not be placed on this evidence. The first argument of Nassonov is purely a negative one and has little or no value. The secretory property of the contractile vacuole similar to that of the Metazoan Golgi apparatus also is not of much value. The view of Gatenby and King seems to be more reasonable, namely, that, since the close association of the Golgi apparatus with the centrioles is almost universal amongst the Metazoa including the sponges, "the Golgi apparatus arose in some primitive flagellated organism in direct association with the blepharoplast." On the other hand we must remember that the Golgi apparatus in animal cells has been shown to consist of vacuoles (Parat) and the contractile vacuole of ciliates may represent the Golgi apparatus.

"King and Gatenby (*Quart. Journ. Micr. Sci.* April 1926) have discovered

certain osmiophile and pyriform structures in the Ciliate *Opalina ranarum* which they consider as the Golgi apparatus. In many cases the cilium can be followed very clearly from outside into the cortex, through the cortex into the sub-cortical region full of non-osmiophile granules, into the inner region of *Opalina*, where it may be seen distinctly to join the pyriform body (Golgi apparatus). These observations and those of Duboscq and Grassé (*C. R. Soc. Biol.* Feb. 1925), who consider the parabasal body of flagellates to be the homologue of the Golgi apparatus of higher forms, go strongly against the 'contractile vacuole' theory of Nassonov."

Dictyokinesis or the behaviour of the Golgi elements during mitosis.

During dictyokinesis the behaviour of the dictyosomes is somewhat variable, although they are always sorted out into two more or less equal parts. In the simplest case, *e.g.* the segmenting eggs of Pulmonates (Gatenby, 1919), there is a purely passive and mechanical distribution of the diffuse dictyosomes into two approximately equal halves, without any definite orientation either with regard to the poles or the equator of the spindle. In other cases, however, the dictyosomes seem to be influenced by the mitotic figure.

In the spermatocyte divisions of Molluscs and Mammals (Gatenby and Ludford, 1921) the localised type of Golgi apparatus divides into two parts which pass to the poles of the spindle and during metaphase spread out throughout the cytoplasm and become localised again in the two resulting cells. A somewhat similar type of dictyokinesis has been recently described by Wilson (*Am. Nat.* Nov.–Dec. 1925) in *Opisthacanthus*. In other cases, *e.g.* the spermatocytes of insects (Bowen), the localised type of Golgi apparatus first breaks up into separate batonettes that arrange themselves round the equator of the spindle. They are then sorted into two halves that move towards the opposite poles of the spindle while the chromosomes are still in the metaphase. At the close of cell division the dictyosomes again aggregate in each cell and give rise to the localised type of Golgi apparatus. This type is beautifully illustrated by the abnormal tripolar spermatocyte divisions of *Chlorochroa* (Bowen, 1922), in which the dictyosomes separate into three groups in connection with the three poles of the mitotic figure while the chromosomes are still in the metaphase and long before the division furrow has appeared. This clearly shows that the sorting out of the dictyosomes is not a mechanical result of division furrow but is influenced by the centrioles.

Function.

It has been abundantly made clear during the last decade that the Golgi apparatus has a definite rôle to play both in the germ cells and the somatic cells of the secretory type.

The pioneer researches of Gatenby (1917) and Bowen (1920 and later) have proved that the acrosome, or the boring apparatus of the sperm, arises in association with the Golgi batonettes, now called the acroblasts. The acroblasts may remain separate from each other, *e.g.* Lepidoptera (Gatenby and Bowen), or they may fuse

with each other to form a single compact acroblast as in Hemiptera (Bowen), Coleoptera (Bowen) and, to some extent, in *Lepisma* (Bowen) also, and Mammals (Gatenby and Woodger). The older literature is full of the most fantastic accounts of the origin of the acrosome. For instance, Montgomery (1911), working on the Hemipteran *Euschistus*, describes the origin of the acrosome from a body called the "sphere" or the "idiozome" which we now know to be the Golgi apparatus whose chromophilic rim is destroyed by the technique used.

Gatenby (1917), working on the Lepidoptera, was the first worker who gave, on the whole, an accurate account of the origin of the acrosome, although the later work of Bowen on a number of insect groups varies somewhat in detail from the earlier work of Gatenby. According to Gatenby each acroblast secretes a "dark dot," the acrosomal granule. Later "the acrosome is finally formed by the running together of several acroblasts." Bowen's later work on Lepidoptera, Hemiptera, Orthoptera and Coleoptera has made it abundantly clear that the acrosome is not directly formed from the acroblasts (Golgi elements) but is a secretory product thereof. For instance, in the Lepidoptera each acroblast secretes an acrosomal vesicle in which is differentiated a darkly staining body, the acrosomal granule. The acroblasts themselves pass into the tail and are cast off along with other detritus, while the acrosomal vesicles, each containing an acrosomal granule, fuse together and give rise to the acrosome.

In Mammals Gatenby and Woodger (1921), building on the earlier work of Moore (1893) and several other workers, have shown in *Cavia* that the acrosome arises by the fusion of pro-acrosomic granules differentiated in the Golgi-idiosome complex. Around each pro-acrosomal granule, comparable with the acrosomal granule of insects, a vacuole is produced which is strictly comparable with the acrosomal vesicle of insects. The most interesting Da Fano preparations of the guinea-pig by these workers, which I personally examined at Dublin, also show clearly that the bead on the middle piece of the sperm earlier figured by Retzius is derived from the Golgi apparatus.

To the best of my knowledge the origin of the acrosome from the Golgi elements is most clearly demonstrated in Insects and Mammals only, but in other cases, *e.g.* scorpions (see Gatenby and Bhattacharya, *La Cellule*, Jubilee number), *Lithobius* (see Nath, 1925 a) and *Peripatus* (see Gatenby, 1925), one can see the Golgi elements stuck to the nuclear membrane of the maturing sperm, but it is not possible to study the various stages of acrosome formation as seen in Insects and Mammals. We cannot, of course, expect that every material would be as favourable as the Insects and Mammals but we are probably justified in concluding, on the analogy of Insects and Mammals, that the Golgi origin of the acrosome is a universal process in the animal kingdom.

The old idea that the acrosome is a perforatorium to enable the sperm head to pierce the egg membrane (Waldeyer) seems to be irreconcilable with the broad cap-like acrosome of the mammalian sperm and also with the position of the acrosome in certain sperms recently studied by Bowen (1924). In the beetles, *Chelymorpha* and *Lixus*, the acrosome lies along the whole length of the sperm head. In Hemi-

ptera the bulk of the acrosome is spread out along one side of the head and finally in *Lepisma* the centriole passes to the anterior tip of the nucleus and the axial filament grows backwards from it, but the acrosome remains in the neck region, sending backwards a long filamentary outgrowth alongside the axial filament into the tail. "An acrosome pointed backwards into the tail region is as complete a *reductio ad absurdum* of the 'cutting-tool theory' as could be reasonably hoped for" (Bowen, 1924, p. 387). In such atypical cases it is difficult to understand how the acrosome could function as the perforatorium and, further, the complexity of the process by means of which the acrosome arises seems to be incompatible with the boring function, a function which could be easily performed by the pointed end of the nucleus. The broad cap-like acrosome of the mammalian sperm, the exceptional cases of *Lepisma* and others, and finally the fact that, in the process of fertilisation, "the egg actively co-operates and may actually be regarded as engulfing the spermatozoon" (Cowdry, 1924, p. 456), seem to discountenance the "perforatorium" theory of the acrosome. What other function is to be ascribed to the acrosome it is impossible to suggest with certainty in the present state of our knowledge, but Bowen (1924) has suggested an extremely interesting possibility that the acrosome, since it is a secretory product of the Golgi-idiosome complex, may represent the "sperm receptors" postulated in Lillie's theory of fertilisation. In this connection it might be of some interest to mention that the only case in which the acrosome has been traced into the egg is that of *Nereis* (Lillie, 1912). After entering the egg it breaks up into a number of deeply staining granules which persist for some time in close contact with the sperm nucleus and later disappear.

The problem of the relationship of the Golgi apparatus with secretory processes has been recently brought into prominence by the valuable researches of Nassonov (1923, 1924), Bowen (1923, 1924, 1926) and Ludford (1925). We are not yet in a position to settle this question finally but the very circumstantial and, on the whole, concordant accounts which these writers have given leave little doubt that the Golgi apparatus takes a prominent part in secretory processes. In addition to the fact that a very close topographic relationship exists between the Golgi apparatus and the secretory granules, Nassonov (1923) and Bowen (1924) have adduced evidence that the secretory granules are actually products of the Golgi material in the glands of the salamanders.

Nassonov (1923), working on the various glands of salamanders with Kolatchev's improved osmium technique, has demonstrated that the secretory granules first make their appearance in close contact with or actually embedded in the meshes of the Golgi network. Each secretory granule, when it severs its connection from the Golgi reticulum, carries with it a cap of osmiophile material which is impregnated exactly like the apparatus by osmium tetroxide and which Nassonov interprets as a portion of the apparatus. Later, the secretory granules pass into the general cytoplasm where they increase in size. Finally, they dissolve and pass as liquid secretion into the lumen of the gland. Nassonov does not altogether deny that the mitochondria may also play some part in the process of secretion, perhaps as an intermediary between the dissolved material of secretion and the actual

secretory granules formed under the influence of the Golgi apparatus. Bowen (1923, 1924), working on the same material, has confirmed Nassonov's account in its most essential aspects, but he is not inclined to ascribe any secretory rôle, however insignificant, to the mitochondria. Bowen invites attention to the very close similarity that exists in the appearances of a secretory granule attached to a girdle of Golgi material and the acrosomal vesicle with the acroblast of Lepidopteran sperms. In a recent paper, Bowen (*Quart. Journ. Micr. Sci.* April 1926) has also shown in the oil gland of the chick that each secretory granule is very closely associated with each Golgi element. He did not find these discrete Golgi elements lying free in the cytoplasm. These observations strongly support the view that the secretory granules arise by the synthetic activity of the Golgi apparatus. In the same paper Bowen shows a close topographic relationship between the secretory granules and the Golgi network in the white portion of the inguinal gland of rabbit. Nassonov (1924), working on the epididymis, also shows a very close topographic relationship between the Golgi apparatus and the secretory granules which are of one kind only, as in the salamanders. Ludford (1925), however, in an excellent paper, has studied the epididymis in much greater detail by means of his own improved osmium technique (1924 and 1925). He describes a marked hypertrophy, as others have done, of the Golgi apparatus during secretory activity and the secretory products make their appearance in intimate relationship with the apparatus in the form of

(i) "Droplets," which are the only kind of secretory products described by Nassonov.

(ii) "Granules," also described by Fuchs (1902), but interpreted by Nassonov as modified mitochondria, which in contact with the Golgi apparatus are undergoing transformation into the secretion. Ludford, however, has shown that the mitochondria are quite distinct from these "granules."

(iii) "Complex granules," probably consisting of a solid core and a fluid periphery. Such "complex granules" have been described by Nassonov (1924) in the epithelial cells of the seminal vesicle and also by Bowen (1926) in the parotid of the cat.

Ludford also describes nucleolar extrusions and nuclear budding resulting in the formation of secondary nuclei, also described by Benoit (1921). The mitochondria also increase in number at the onset of secretory activity, and decrease during the course of secretion. Often they are found in immediate contact with the formed secretion. Ludford, therefore, calls attention to the very important point that "undoubtedly, as in oogenesis, so in secretion, each of the cell organs contributes its part." "The cell as a whole is a functioning unit, and we are probably approaching nearest to the truth in studying the changes occurring in its visible structure, rather than seeking to attribute substances formed by it to the activities of any one of its component parts."

Bowen (1926) has published a large paper on the glands associated with the alimentary tract, mostly of Vertebrates. In all the glands that he has studied, namely, parotid and sub-maxillary glands and liver and pancreas of cat, pancreas of certain salamanders and the salivary glands of *Limax*, he has shown a very close

topographic relationship between the Golgi apparatus and the secretory granules. At the onset of secretory activity the juxta-nuclear Golgi apparatus undergoes a marked hypertrophy. The important contribution, however, that Bowen has made is the difference in the nature of the secretory granules of the cells of the serous and the mucous types which is correlated with the difference in the behaviour of the apparatus.

The parotid gland (cat) consists entirely of serous cells. Each secretory granule which makes its first appearance in the meshes of the Golgi network consists of a clear vesicle. Later a granule appears within this vesicle, which eventually fills or nearly fills the limits of the old vesicle. The granule grows in size apparently at the expense of the material of the vesicle. Such granules of duplex nature have been described by Altman (1894), by Ludford (1925) in the epididymis, and by Nassonov (1924) in the epithelial cells of the seminal vesicle. This type of secretory granule recalls to mind the acrosmal vesicle with the granule of the sperm-forming cells of the insects. Closely related with the long time which these granules take in maturing the Golgi apparatus ramifies throughout the cell and is spun out into very thin strands reaching to every granule and effecting the simultaneous ripening of the whole batch.

The submaxillary gland consists of both serous and mucous cells. The history of the secretory granules and the Golgi apparatus in the serous cells is similar to that of the serous cells of the parotid. But in mucous cells the secretory granules are not of duplex nature and are completed very soon after their dissociation from the Golgi apparatus. Hence the apparatus does not ramify throughout the cell but maintains a more compact location. If these observations are extended, it should be easy to find out whether a cell is of the serous or the mucous type by the type of Golgi apparatus present in it.

In these glands which Bowen has studied the nucleus undergoes degenerative changes and does not seem to take part in the secretory processes.

The accounts of Nassonov, Ludford and Bowen become all the more convincing when it is remembered that in these glands the Golgi apparatus lies next to the lumen, between it and the nucleus. Parat who has studied the origin of secretory granules in the pancreas of fish and Amphibians which had been vitally injected with neutral red and post-vitally with Janus green strongly supports the view that the secretory granules are derived from the Golgi apparatus which, however, is not in the form of a network but consists of a number of vacuoles. In the words of Parat himself—"Le vacuome est seul producteur de grains de sécrétion; il n'y a pas transformation directe du chondriome en ces grains; la présence du chondriome est sans nul doute aussi indispensable que celle du noyau, mais son rôle reste inconnu." (*Comp. rend. des séances de la Soc. de biol.* Jan. 17, 1925.)

The view that the Golgi apparatus takes an active part in secretory processes is further supported by the work of Gatenby and his pupils, and, more recently, of the present writer on the behaviour of the Golgi apparatus in the vitellogenesis of certain animals.

Gatenby and Woodger (1920), Ludford (1921) and Brambell (1924) have made

a very careful study of vitellogenesis in *Patella* and their concordant accounts seem to have established clearly that in this form yolk, which is fatty in nature, arises in association with the Golgi apparatus.

In the young oocyte the Golgi apparatus consists of the usual batonettes lying on the archoplasmic sphere. As the cell grows some of the rods spread out in the cytoplasm, while the sphere and the pieces of archoplasm probably attached to each of the separate rods become loaded with fat and represent the initial stages of yolk formation. The individual elements divide rapidly as they spread out in the cytoplasm and lead to the formation of yolk spheres in the following manner. In many places several Golgi rods collect together and the cytoplasm between them becomes more stainable under their influence. Gradually this stainable cytoplasm swells up into a yolk sphere with the Golgi rods applied to its surface and almost completely surrounding it. This process of yolk formation continues throughout oogenesis. As each yolk sphere is completed, the majority of the surrounding batonettes break away from it and fragmenting pass, either to the periphery, where they form a layer under the vitelline membrane, or towards the nucleus, around which they form another layer. Some, however, of the batonettes remain attached to the yolk spheres (Brambell).

In *Helix aspersa* Brambell (1924) has shown that the yolk, which is fatty in nature, arises by a direct metamorphosis of the Golgi rods. This worker has performed interesting centrifuging experiments on the eggs of *Patella* and *Helix* and has clearly shown that the upper "grey" layer of conklin is the "Golgi yolk" which is fatty in nature, the lower "yellow" layer is made up of "swollen mitochondria" impregnated with some yellow pigment, and the middle clear area consists of cytoplasm.

In the centrifuged egg of *Saccocirrus* Gatenby (1922) describes a heavy nucleolar deutoplasm which forms the lower pole and a light fatty yolk which forms the upper pole and which is "probably of the Golgi elements." King (1924) and Nath (1924) have shown that in the eggs of *Lithobius* there are two kinds of yolk, fatty and albuminous, exactly like the egg of *Saccocirrus*. The albuminous yolk seems to be in some way associated with nucleolar extrusions of a remarkable type; and Nath has further shown that the fatty yolk is formed by a direct metamorphosis of the Golgi elements and forms the upper pole in the centrifuged egg, whereas the lower pole is formed by the proteid yolk. The juxta-nuclear Golgi apparatus of the young oocyte, consisting of small batonettes, spreads out throughout the cytoplasm and fragmenting gives rise to small fatty granules which grow in size and form the fatty yolk. There are all possible gradations between a Golgi batonette and a fatty granule and the conversion of the former into the latter can be seen with almost diagrammatic clearness. To study this process the prolonged osmication method is indispensable as the method would fix both the Golgi batonette and the fatty yolk. King, who is doubtful about the origin of the fatty yolk, did not, unfortunately, use this method. She used the Da Fano method which would not fix fat and Flemming-without-acetic which would not fix the Golgi batonette.

In the eggs of spiders Von Bambeke (1898) had described two kinds of yolk, fatty and albuminous. The latter arises *de novo* in the cytoplasm and the former arises directly from the "yolk nucleus" or the "pallial substance." This substance

is present in the form of a crescent-shaped body near the nucleus and, with the growth of the oocyte, it spreads throughout the cytoplasm and forms the fatty yolk. The present writer has shown in a paper not yet published that the "yolk nucleus" of Von Bambeke consists of granular mitochondria in which are embedded the Golgi crescents or rings. Indeed, it is remarkable that in spite of unsatisfactory technique Bambeke shows granules (really mitochondria) and rings (really Golgi elements) in his "yolk nucleus," although he could not identify them. The Golgi apparatus had not yet been discovered in those days. I have proved that the Golgi elements are directly metamorphosed into the fatty yolk by a process of loading up of the Golgi element as well as the sphere substance with fat. It is impossible to decide, however, whether this fat comes from the cytoplasm or from the Golgi material which may undergo a chemical change (cf. *Helix, Lithobius* and, probably, *Saccocirrus*). I have been able to confirm Bambeke's statement that the albuminous yolk arises *de novo* in the cytoplasm. Similarly the writer with Hussain and with Mehta has shown (unpublished) in *Scolopendra* and the firefly *Luciola* respectively that the fatty yolk in these eggs, as distinct from the albuminous yolk, arises directly from ring-like or vacuole-like Golgi elements. The whole process of the swelling up of the Golgi elements into fatty yolk spheres can be studied with diagrammatic clearness. In a recent paper King (*Proc. Roy. Soc.* June 1, 1926) also describes a swelling up of the Golgi element into fatty yolk.

The important point should be noted that in *Patella, Helix, Saccocirrus, Lithobius* and Spiders, etc., in which yolk originates directly or indirectly from the Golgi elements, such yolk is always fatty in nature. There does not seem to be any doubt that such highly metamorphosed Golgi elements are used as yolk in embryogeny.

In several other cases, however, *e.g.* Ascidians (Hirschler, 1915), *Buthus* (Nath, 1925 *b*), *Julus* (Nath, in press), the Golgi elements simply swell up without in any way changing their chemical nature and it is very likely that in such forms the swollen Golgi elements will shrink to their normal size during embryogeny and will not be used as yolk. The possibility, however, remains that when such swollen Golgi elements shrink, some of their material will pass into the cytoplasm and may be used up in the metabolism of the cell.

It is difficult to deny the secretory function of the Golgi apparatus in gland cells, in certain cases of vitellogenesis and in the formation of the acrosome. We must not, however, hasten to the conclusion that the function of the Golgi apparatus is always secretory, since it is present in non-glandular cells also, such as nerve cells, muscle cells, etc.

MITOCHONDRIA.

Discovery.

It is impossible to say who exactly discovered mitochondria. Long before they were brought into prominence by the researches of Meves, Regaud, Fauré-Fremiet, Guilliermond, Bensley, Cowdry, Gatenby and several others, they were imperfectly fixed and known by different names such as "cytomicrosomes" (La Vallete St George and Strasburger), "fila" (Flemming), "bioblasts" (Altman), "plastidules"

(Maggi and Zoja brothers), Boveri's "archoplasmic granules" which in the seg-menting egg of *Ascaris megalocephala* surround the centrosome, "interstitial korner" (Koilliker), "neurosomen" (Held) and "granules," "Nebenkerns," etc.[1] In plants the mitochondria were first discovered by Meves (1904). Benda dis-covered them in 1897 in many kinds of cells, especially the sperm-forming cells, and named them mitochondria.

Form.

Microchemically the mitochondria show distinct characters but they are highly plastic and polymorphic bodies, their form varying not only in different cells but even in the same cell at different times. They may be granular or filamentous, or the granules may be arranged in a linear series; or the filaments may form a network, especially round the nucleus; or they may give rise to a massive body in the sperm-forming cells, the "mitosome" or the "Nebenkern," which may assume complicated patterns as in Insects; or the granular mitochondria may fuse to form "chondrio-spheres" as in the scorpion, *Opisthacanthus* (Wilson) or, lastly, they may form a ring-shaped body as in *Centrurus* (Wilson).

In spermatogonia and young oocytes the mitochondria are frequently aggre-gated near the nuclear membrane and round the centres but never so definitely as the Golgi apparatus. This led various earlier observers, working on oocytes, to regard this juxta-nuclear substance as nuclear in origin (yolk nucleus) but later researches have proved in many cases that it consists of mitochondria, *e.g. Lum-bricus*[2] (Harvey, 1925), or both mitochondria and Golgi apparatus, *e.g.* spiders (Nath).

This highly plastic nature of the mitochondria has been established not only by the study of fixed cells but also by the study of living cells in tissue-culture (Lewis and Lewis, 1914 and 1915). These writers describe movements of the mitochondria in living cells and also rapid changes in their shapes. The granules may become arranged in a linear series or the individual granules be converted into filaments which can give rise to complicated networks.

Composition, technique and physical consistency.

Direct analysis of the mitochondria being obviously very difficult, their chemical composition, as in the case of the Golgi apparatus, can be inferred only by their reactions to reagents. They are fully or partly soluble in acetic acid, ether, alcohol, chloroform and other fat-solvents unless they are previously treated with osmic acid, chromic acid, etc. They can reduce osmium tetroxide and become black but not so intensely as the Golgi apparatus. It has therefore been suggested, especially by Regaud (1908), working on Mammals, Fauré-Fremiet (1910 c), working on Protozoa, and Lowschin (1913, 1914), working on plants, that the mito-chondrial substance is a chemical compound consisting of phospholipins and proteids and are therefore of the nature of phosphatids, such as lecithin. The

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[1] See Duesberg (1912) for earlier literature.

[2] In this form the Golgi apparatus is also of the localised type and not of the diffuse type as claimed by Harvey. (See Gatenby and Nath.)

proportion of these two substances seems to vary in different cells and also in the same cell at different stages, as shown by the effect of acetic acid. Regaud (1910) discovered a progressive increase in the resistance of the mitochondria to acetic acid from spermatogonia to spermatozoa. The present writer has observed a similar thing in the spermatogenesis of the scorpion (*Palamnaeus*). N. C. Nicholson (1916) reports that the mitochondria in different kinds of nerve cells differ in their solubility to acetic acid. The microchemical nature of the mitochondria, however, is much more constant than their form. Indeed, we identify mitochondria mostly by their microchemical reactions.

Mitochondria appear to be solid or semi-solid bodies. Their refractive index is higher than that of the Golgi apparatus. Chambers (1915) has made a microdissection study of the mitochondria in the spermatocytes of the grasshopper. "In the fully-grown primary spermatocyte, they are minute and highly refractive granules and threadlets, which are uniformly distributed in the hyaline cytoplasm immediately around the nucleus. During life they appear to be constantly shifting in position and to be disappearing and reappearing in the cytoplasm." They are brilliantly illuminated by dark-field illumination and are also visible by transmitted light, although not so prominently.

Mitochondria can be stained *intra vitam* by Janus green B. Janus green (Grübler) and Janus green C will not do, although "these dyes differ only in the substitution of an H_2 and $(CH_3)_2$ group in place of the $(C_2H_5)_2$." It should be used in very dilute solution, about 1 : 10,000 or 1 : 25,000 as recommended by Cowdry. Since it penetrates very slowly, the cells should be carefully teased to enable the stain to reach the mitochondria. Permanent preparations can be made by Benda's, Altman's, Bensley-Cowdry's, Regaud's, Champy-Kull's or Flemming-without-acetic methods. The osmium and the silver methods, so useful for the study of the Golgi apparatus, may sometimes show the mitochondria but not satisfactorily.

Distribution.

"Mitochondria have been found to occur in representative organisms ranging from man to the Protozoa and from the Angiosperms to the Fungi and Myxomycetes. They have also been observed in certain Algae and Diatoms (Guilliermond, 1921) but their existence is doubtful in Bacteria" (Cowdry). Their existence therefore is almost universal. Further research may bring to light cases in which there are no mitochondria but this seems to be unlikely. In senescent cells, *e.g.* the non-nucleated red blood corpuscles, the mitochondria disappear with the appearance of haemoglobin, which has given rise to the belief that the mitochondrial material is converted into haemoglobin.

Mode of origin.

Some cytologists believe that mitochondria do not arise *de novo* in the cytoplasm but always by the division of pre-existing ones (Guilliermond, 1914, Moreau, 1914, Terni, 1914, etc.). Fauré-Fremiet (1910) claims that in the living ciliates the mitochondria divide along with the nucleus. The evidence which these and other writers

who believe in the autonomy of mitochondria have furnished is rather dubious, because what may be interpreted as a division stage may be two mitochondria that have come into close contact. On the other hand the study of living cells in tissue-cultures (M. R. Lewis and W. H. Lewis, 1914, 1915) has shown that filamentous mitochondria may break up into granules. Due allowance, however, should be made in this case for the abnormal condition in which the cells live. Such a frag-mentation of the mitochondria has been recently described by Wilson (*Am. Nat.* Nov.–Dec. 1925) in the scorpion (*Opisthacanthus*). At the most we can say that mitochondria can divide but the statement that the mitochondria can never arise *de novo* is not warranted, especially when Chambers (1915) reports that the mito-chondria in the spermatocytes of the grasshopper may appear and disappear under the very eye of the observer. On this question Cowdry (1924) cites a test case: "Among the Protozoa, the piroplasms pass through a stage of development within the red blood corpuscles of Vertebrates in which the cytoplasm is very much reduced and *Anaplasma marginale* is said during this phase to consist wholly of nuclear material. It would be a nice problem to ascertain whether with increase of cytoplasm mitochondria appear, because, if so, it would be a clear case of their *de novo* origin."

Chondriokinesis.

There is ample evidence, however, that mitochondria do divide in some cases of cell division. But here again their division appears to be the mechanical result of division furrow and does not prove that mitochondria are independent bodies, capable of self-perpetuation. There are two principal types of chondriokinesis. In the first type the mitochondria are sorted out passively into two groups without any orientation either towards the poles or the equator of the spindle and without themselves undergoing division. This type is illustrated by the germ cells of Vertebrates (Benda, 1903; Duesberg, 1910), by the segmenting ova of Pulmonates (Gatenby, 1919), by many somatic cells and by the germ cells of scorpions (Wilson) *Opisthacanthus, Vejovis, Hadrurus*, in which the big "chondriospheres" of the spermatocyte are sorted out into two nearly equal groups, without any division of the individual mitochondria. In *Ascaris* (Hirschler, 1913) the mitochondria are attracted towards the centres but here also the mitochondria do not divide.

The second type of chondriokinesis can be illustrated by most insects, *e.g.* Coleoptera (Benda, 1903; Duesberg, 1910), Hymenoptera (Meves, 1907 *a*), Hemiptera (Bowen, 1920, and earlier workers) and Orthoptera (Payne, 1916), and has also been studied in the living spermatocyte of the grasshopper (Chambers, 1915) and by Lewis and Robertson (1916). In this type the elongated mitochondria place themselves parallel to the spindle and closely surround it. At the time of cell-constriction most, if not all, of the mitochondria are cut across. In *Paludina* (Gatenby, 1918) there are four to six rod-like mitochondria which are cut across in the same way as in Insects. In the scorpion, *Centrurus* (Wilson), "all the chon-driosome material aggregates into a single ring-shaped body, which is placed tangentially to the spindle in the first spermatocyte and is cut across transversely by the division accurately into two half-rings. Each half-ring now breaks apart

to form two parallel rods which in the second mitosis are again cut across transversely into two shorter rods. The original ring is thus divided into eight equal parts, of which each resulting cell (spermatid) receives two, a process comparable in precision with the division of a heterotypic chromosome, though very different in detail."

Function.

The rôle of the mitochondria in histogenesis has been the subject of controversy for many years, without having led to any general agreement among cytologists. In 1897 Benda suggested a definite rôle for them both in fertilisation and histogenesis. He regarded the mitochondria as definite, permanent and autonomous cell-organs, identical with the "bioblasts" of Altman, from which during histogenesis various specialised cell structures may arise. This suggestion of Benda was elaborated by Meves who has published a large number of papers in support of Benda's general conclusions and he has been supported by the important work of Fauré-Fremiet, Hoven, Duesberg, Regaud, Guilliermond and several others to be mentioned below.

Meves found a large number of mitochondria in all kinds of embryonic cells in Vertebrates and he noticed a great decrease in their numbers when histogenesis was well advanced. He argued that the mitochondria of the embryonic cell give rise to a large number of formed bodies in the specialised cells such as myofibrils of both smooth and striated muscle cells, neurofibrils, collagenous fibrils of cartilage, connective tissue fibrils, the basal filaments of ciliated cells, the fibrillae of glandular epithelia and various types of granules such as secretory granules, leucocytic granules, pigment granules and various forms of yolk spheres. A list of eighty substances in the formation of which mitochondria are said to take part was published by Cowdry in 1918 and to this list some more have been added. Very few of these conclusions have not been contradicted by other observers.

With regard to the myofibrils Meves (1907 *a*, *b*, 1909) believes that the chondriosome elongates and directly gives rise to the myofibril—a view strongly supported by Duesberg (1909), Luna (1913) and others. Cowdry (1918), however, is opposed to this view, but so far no specific objections have been made against the view of Meves and Duesberg. Gaudissart (1913) shows that the myofibril is not a modified filamentous chondriosome as claimed by Meves and Duesberg, but "that the primary basis is furnished by the plasmatic reticulum with which the chondriosomes cooperate in building up the fibril" (Sharp, 1921).

Hoven (1910) and Meves have also shown that the neurofibrils and the collagenous fibrils of cartilage are derived from the chondriosomes, but this view again is contradicted by Cowdry (1914) and others.

Regaud (1911), Guilliermond (1914), Hoven (1910, 1911), and Lewitski (1914) believe that the secretory granules may in some cases arise from the mitochondria. Nassonov (1923) and Ludford (1925), who give a very circumstantial account of the origin of the secretory granules from the Golgi network, do not altogether deny that the mitochondria may also be concerned in this process. Nassonov admits the possibility that the mitochondria may play the part of an intermediary between the

dissolved secretory material and the actual secretory granules formed under the influence of the Golgi apparatus. According to Ludford there is an increase in the number of mitochondria at the onset of secretory activity and a decrease during the course of secretion. Often the mitochondria are found in immediate contact with the formed secretion.

That mitochondria take some part in the production of the secretory granules seems to receive some support from their arrangement within the glandular cells.

For example, in the kidney the mitochondria are often most numerous in the basal region next to the blood vessels. A similar arrangement obtains in all other glands with fixed polarity in which the direction of secretion is proximo-distal, that is to say, from the blood stream to the lumen of the duct. Champy has found that mitochondria are arranged in epithelial cells of the intestine where they tend to accumulate at both poles of the cell. This he believes to indicate the existence of a double polarisation in two directions, for secretion and for absorption. It is interesting to note that Bensley (1916) is of the opinion that the original proximo-distal polarity of thyroid cells has been reversed and points to the heaping-up of mitochondria next to the lumen instead of near the peripheral blood vessels as one indication of a change in the direction of secretion. It is, therefore, possible that the mitochondria may serve to some extent as indicators of secretory polarity, like the Golgi apparatus (Cowdry).

Van der Stricht (1905, etc.) and Lams and Doorme (1908), working on Mammals, Loycz (1909), working on Ascidians (*Ciona, Molgula, Ascidia*), Hirschler (1915), working on *Ascidia*, and Shaffer (1920), working on *Cicada*, report the origin of yolk from the mitochondria. In a recent paper Brambell (*Phil. Trans. Roy. Soc. Lond.* Series B, Vol. 214) shows that in the egg of the fowl some of the yolk arises directly from the mitochondria. Similarly in *Lymnoea, Patella and Helix* Gatenby and his pupils show a swelling of the mitochondria which, however, as shown by Gatenby (1919) in *Lymnoea*, shrink to their original size in embryogeny. A similar shrinkage of the swollen mitochondria is reported by Hirschler in *Ascidia*. Dubreuil (1913) and Murray (1919) consider that fat in some cases originates from the mitochondria.

It must be admitted that the whole subject of mitochondrial histogenesis is in a very doubtful position. There are a few substances that are not said to arise from the mitochondria and this fact seems to have thrown into discredit the whole subject of mitochondrial histogenesis. If the mitochondria are phosphatids as we have some reason to believe, we can understand their direct transformation into fatty or albuminous yolk, secretory granules, etc., but "that masses of this material should bodily transform into haemoglobin which contains iron, chlorophyll which contains magnesium, and the colloid of the thyroid gland with its iodine is unlikely" (Cowdry). It is true that the mitochondria are abundant in the embryonic cell as emphasised by Meves and others, and further they disappear or are much reduced in numbers when the various formed bodies arise in the specialised cell. This, however, does not mean, unless some other evidence is given, that the mitochondria directly give rise to the formed bodies. All that we can at present say with certainty is that the mitochondria play some very active part, like the Golgi apparatus, in the general metabolism of the cell.

In plants the most prominent view concerning the mitochondria is that they are directly transformed into plastids. This view was first suggested by Lewitski (1910) and later supported by Forenbacher (1911), Pensa (1914), Cavers (1914) and others. Guilliermond's (1911–1920) exhaustive researches show that the mitochondria are transformed directly into amyloplasts, chloroplasts, chromoplasts and elaioplasts, which form respectively starch, chlorophyll, anthocyanin and fat. In the embryonic cells the mitochondria are abundant but when the cell differentiates their number is greatly reduced, and Guilliermond, Meves and others have traced all stages between a chondriosome and a plastid. This conclusion has been denied by Lundegardh (1910), Meyer (1911), Rudolph (1912), Lowschin (1913, 1914), Scherrer (1914), Von Derschau (1914), Von Winiwarter (1912), Sapéhin (1915) and Mottier (1918) and others. Some of these writers hold that the embryonic plastids are from the beginning quite distinct from the mitochondria, although they admit that in the embryonic cells it is not always possible to distinguish the smallest plastid from the mitochondria. If it can be proved conclusively that plastids, which are, in some cases at least, capable of division, are derived from the mitochondria, we have then an indirect evidence in favour of the autonomy of the mitochondria.

In a recent paper (*Am. Nat.* March–April 1926) Cowdry, reviewing the evidence furnished by various workers in favour of the origin of the plastids from mitochondria, considers it highly probable that "the mitochondria, with the assistance of the cytoplasm, change into the larger and structurally more complex plastids."

Closely connected with the view that the plastids are directly derived from the mitochondria is the eclectosome theory of Regaud (1909), according to which the mitochondria act as plasts, selecting substances from the surrounding cytoplasm and transforming them in their interior into various products. There is ample evidence that mitochondria may contain starch, colouring matters and various other substances in plant cells and even in animal cells (*e.g.* the eggs of *Lymnoea*), but this may be due to the peculiar chemical and physical nature of the mitochondria rather than to the supposition that they act as plasts. In this connection see Cowdry (*Am. Nat.* March–April 1926).

It has also been suggested by Kingsbury (1912) and Mayer, Rathery and Schaeffer (1914) that mitochondria play a part in protoplasmic respiration. They contend (1) "that mitochondria are phosphatids containing unsaturated fatty acids with etylidene groups, and are therefore chemically well adapted to function in oxidations and reductions, and (2) that agents which attack lipoids (like alcohol, ether and chloroform among anaesthetics) at the same time cut down respiration" (Cowdry).

Portier (1917, 1918, 1919) in France, and Wallin (1922, 1923) in the United States, have contended that mitochondria are symbiotic bacteria, somewhat like the "bioblasts" of Altman, that have become adapted to an intracellular existence. Wallin contends that this symbiotic existence of the bacteria in the cytoplasm of higher organisms "had its inception at the dawn of phylogenetic evolution." He further says that during this very long period the symbiotic bacteria will become different from free-living ones, and he is inclined to interpret some of the numerous

differences that exist between mitochondria and bacteria on this basis. This theory, to my knowledge, has met with no support from any responsible quarter and it would be futile to discuss it in detail here.

If mitochondria are symbiotic bacteria it should be very easy to see them multiplying in the cytoplasm of higher organisms but it is not really so. Wallin is trying to grow mitochondria independently in cultures and he hopes to establish that they can multiply like bacteria. Obviously this would be the most crucial test of his theory.

The history of the mitochondria in many cases of spermatogenesis is opposed to Wallin's theory. It is difficult to understand how mitochondria, if they were symbiotic bacteria, could unite to form the mitosome in the spermatid and undergo such complicated morphological changes as they do in the formation of the tail sheath of the insect sperm. For other criticism of Wallin's theory reference may be made to Cowdry (1923 *a* and 1923 *b*), Nicholson (1923), Cowdry and Olitsky (1922), and Trojan (1919).

The idea of cytoplasmic heredity came into prominence with the well-known experiments of Godlewski (1906). Meves (1911), who discovered the interesting case of *Ascaris megalocephala* in which the sperm mitochondria enter the egg and live and proliferate there, was led to consider the mitochondria as the material basis of cytoplasmic heredity. No evidence, however, has been furnished that paternal and maternal mitochondria fuse with each other in the egg.

The objections to Meves' theory are so strong and have been discussed so often that a bare reference to them will suffice here. In the first place the distribution of the mitochondrial material during the meiotic divisions in spermatogenesis is not, in the least, on a par with that of the nuclear material (see Chondriokinesis). Even in the most impressive case of *Centrurus* the mitochondrial ring is cut transversely and not longitudinally. Again, although in *Opisthacanthus* most spermatids receive six mitochondria each, in *Palamnaeus bengalensis* (Gatenby and Bhattacharya) and *Palamnaeus fulvipes madraspatensis* (Nath), the number of mitochondria varies from about three to about 13. I cite these cases of scorpions because the mitochondria in the spermatid arrange themselves into a plate at the base of the nucleus and it is extremely easy to count them. Nor does each mitochondrium in these cases divide into two. In oogenesis the polar bodies receive very few, if any, mitochondria.

Secondly, although the mitochondria always[1] contribute towards the formation of some region of the sperm tail, it is by no means certain that the tail always enters the egg. In *Nereis* (Lillie, 1912) and *Platynereis* (Just, 1915), the mitochondrial middle piece does not enter the egg at all.

Thirdly, in echinoids Meves himself (1912, 1914), who traced the sperm mitochondrial middle piece up to the 32-cell stage, discovered that it passes undivided to one cell. Meves (1918), therefore, was driven to make a highly

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[1] In this connection see a review by the present writer in *Quarterly Journal of Microscopical Science*, 1925. Montgomery had claimed that in *Peripatus* sperm there is a complete discharge of the mitochondrial material but Gatenby (1925) has discovered in the neck region of the sperm certain granules which behave microchemically like the mitochondria but their origin is doubtful.

improbable suggestion that the adult develops only from the cell containing the middle piece while the other cells give rise to larval structures that degenerate in the course of metamorphosis. In the bat (Van der Stricht) and the guinea-pig (Lams) the mitochondrial middle piece passes to one of the two blastomeres when the egg divides. In this case also it has been suggested that the cell that does not contain the middle piece gives rise to the trophoblast.

Conclusion.

In the present state of our knowledge it is not possible to make any general statement with regard to the functions either of the mitochondria or the Golgi apparatus. Even if we consider them self-perpetuating bodies in the cytoplasm, which is yet to be finally proved (although the available evidence points towards that conclusion), the possibility remains that they may arise *de novo*. Nor can we possibly regard them as purely passive bodies like yolk spheres as is clearly shown by their grouping round the centrioles and their subsequent distribution, their orientation towards the poles or the equator of the spindle in certain cases of mitosis, their polymorphic form, their activity in secretory processes and, at least in the case of mitochondria, their movements in the cytoplasm (Lewis and Lewis).

BIBLIOGRAPHY[1].

ALTMAN, R. (1894). *Die Elementarorganismen.* Zweite Auflage. Leipzig.
BENDA, C. (1897). "Neuere Mitteilungen uber die Histogenese des Säugetier-spermatozoon." *Verh. d. Phys. Gesell. Berlin.*
—— (1903). "Die Mitochondria." *Ergebnisse der Anatomie und Entwicklungsgeschichte.* (Merkel and Bonnett, Anatomische Hefte, 2te Abtheilung, Wiesbaden.)
BENOIT, J. (1921). "Sur le rôle du noyau dans la sécrétion épididymaire." *C. R. Soc. Biol.*
BENSLEY, R. R. (1910). "On the nature of the canalicular apparatus of animal cells." *Biol. Bull.* **19**
BOWEN, R. H. (1920). "Studies on insect spermatogenesis. I. The history of the cytoplasmic components of the sperm in Hemiptera." *Biol. Bull.* **39**.
—— (1922 *a*). "II. The components of the spermatid and their rôle in the formation of the sperm in Hemiptera." *Journ. Morph.* **37**.
—— (1922 *b*). "III. On the structure of the Nebenkern in the insect spermatid and the origin of Nebenkern patterns." *Biol. Bull.* **42**.
—— (1922 *c*). "V. On the formation of the sperm in Lepidoptera." *Quart. Journ. Micr. Soc.* **66**.
—— (1922 *d*). "On the idiosome, Golgi apparatus, and acrosome in the male germ cells." *Anat. Rec.* **24**.
—— (1922 *e*). "Abnormal mitosis in spermatogenesis." *Biol. Bull.* **43**.
—— (1923 *a*). "The origin of secretory granules." *Proc. Nat. Acad. Sci.* **9**.
—— (1923 *b*). "On the nature of Mitochondria." *Anat. Rec.* **26**.
—— (1924 *a*). "Notes on the formation of the sperm in Coleoptera and Aptera with a general discussion of flagellate sperm." *Journ. Morph.* **39**.
—— (1924 *b*). "On a possible relation between the Golgi apparatus and the secretory products." *Am. Journ. Anat.* **33**.
—— (1926). "Studies on the Golgi apparatus in gland cells. I. Glands associated with the alimentary tract." *Quart. Journ. Micr. Sci.* **70**.

[1] To save space and unnecessary repetition the whole literature on the subject is not given here. Papers on mitochondria published before 1912 will be found in Duesberg's 1912 monograph. Subsequent literature up to July, 1923, will be found in Cowdry's text-book (1924). Papers on the Golgi apparatus published up to July, 1923, will also be found in Cowdry's text-book. In this bibliography important papers published since July, 1923, up to January, 1926, are given. For the sake of convenience the earlier papers are given if they are cited in the text.

BRAMBELL, F. W. R. (1924). "The nature and origin of yolk." *Brit. Journ. Exp. Biol.* **1**.

—— (1925). "The part played by the Golgi apparatus in secretion, and its subsequent reformation in the cells of the oviducal glands of the fowl." *Journ. Roy. Micr. Soc.*

BRAMBELL, F. W. R. and BHATTACHARYA, D. R. (1925). "The Golgi body in the Erythrocytes of the Saucropzida." *Quart. Journ. Micr. Sci.* **69**.

CAJAL, S. R. (1908). "Les conduits de Golgi-Holmgren du protoplasme nerveux, etc." *Trab. Lab. Invest. Biol. Univ. Madrid*, **6**.

CAVERS, F. (1914). "Chondriosomes and their significance." *New Phytol.* **13**.

CHAMBERS, R. (1915). "Microdissection studies on the germ cell." *Science*, N. S. **41**.

COWDRY, E. V. (1914). "The development of the cytoplasmic constituents of the nerve cells of the chick. 1. Mitochondria and Neurofibrils." *Amer. Journ. Anat.* **15**.

—— (1918). "The mitochondrial constituents of protoplasm." *Contrib. Embryol.* (Carnegie Inst. Wash.), **4**.

—— (1923 a). "The independence of Mitochondria and the *Bacillus radicicola* in root nodules." *Am. Journ. Anat.* **31**.

—— (1923 b). "The distribution of Rickettsia in the tissues of insects and arachnids." *Journ. Exp. M.* **37**.

—— (1924). *General Cytology.*

COWDRY, E. V. and OLITSKY, P. K. (1922). "Differences between Mitochondria and bacteria." *Journ. Exp. M.* **36**.

DUBREUIL, G. (1913). "Le chondriome et le dispositif de l'activité sécrétoire." *Arch. d'Anat. Micr.* **15**.

DUESBERG, J. (1909). "Les chondriosomes des cellules embryonnaires du poulet et leur rôle dans la genèse des myofibrilles, avec quelques observations sur le développement des fibres musculaires striées." *Arch. Zellf.* **4**.

—— (1910). "Nouvelles recherches sur l'appareil mitochondrial de cellules séminales." *Arch. Zellf.* **6**.

—— (1912). "Plastosomen, 'Apparato reticolare interno,' und Chromidialapparat." *Ergebn. d. Anat. u. Entwicklungsgesch.* **20**.

—— (1914). "Trophospongien und Golgischer Binnenapparat." *Anat. Anz. Ergänzungshefte*, **46**.

—— (1920). *Contrib. Embryol.* (Carnegie Inst. Wash.), **9**.

FAURÉ-FREMIET, E. (1910 a). "Mitochondries, etc. liposomes." *C. R. Soc. Biol.* **62**.

—— (1910 b). "La continuité des mitochondries à travers des générations cellulaires et le rôle de ces éléments." *Anat. Anz.* **36**.

—— (1910 c). "Étude sur les mitochondries, etc." *Arch. de l'Anatomie Microscopique, Paris.*

FORENBACHER, A. (1911). "Die Chondriosomen als Chromatophorenbildner." *Ber. deut. bot. Ges.* **29**.

FUCHS, H. (1902). "Ueber das Epithel im Nebenhoden der Maus." *Anat. Hefte*, **19**.

GATENBY, J. B. (1917). "The cytoplasmic inclusions of germ cells. 1. Lepidoptera." *Quart. Journ. Micr. Sci.* **62**.

—— (1918). "IV. Notes on the dimorphic spermatozoa of Paludina, etc." *Quart. Journ. Micr. Sci.* **63**.

—— (1919). "V. *Lymnoea.*" *Quart. Journ. Micr. Sci.* **63**.

—— (1922). "X. The gametogenesis of *Saccocirrus.*" *Quart. Journ. Micr. Sci.* **66**.

—— (1925). "A reinvestigation of the spermatogenesis of *Peripatus*" *Quart. Journ. Micr. Sci.* **69**.

GATENBY, J. B. and BHATTACHARYA, D. R. "Spermatogenesis of an Indian scorpion." *La Cellule.* Jubilee number.

GATENBY, J. B. and BRAMBELL, F. W. R. (1923). "On the supposed homology of the Golgi elements of the mammalian nerve cells, and the Nebenkern batonettes of the genital cells of Invertebrates." *Scient. Proc. Roy. Dub. Soc.* **17**.

GATENBY, J. B. and KING, S. D. (1923). "The Golgi bodies of a coccidian." *Quart. Journ. Micr. Sci.* **67**.

GATENBY, J. B. and KING, S. D. (1925). "The nature of the contractile vacuole." *Nature*, Jan. 1, **31**.

GATENBY, J. B. and LUDFORD, R. J. (1921). "Dictyokinesis in germ cells, or the distribution of the Golgi apparatus during cell division." *Proc. Roy. Soc.* **92**.

GATENBY, J. B. and NATH, V. "Lumbricus." *Quart. Journ. Micr. Sci.* (In the Press.)

GATENBY, J. B. and WOODGER, J. H. (1920). "On the relationship between the formation of yolk and the mitochondria and Golgi apparatus during oogenesis." *Journ. Roy. Micr. Soc.* Pt 2.

—— (1921). "The cytoplasmic inclusions of germ cells. Pt IX. On the origin of the Golgi apparatus of the middle piece of the ripe sperm of *Cavia*, and the development of the acrosome." *Quart. Journ. Micr. Sci.* **65**.

GAUDISSART, P. (1913). "Reseau protoplasmique et chondriosomes dans la genèse des myofibrilles." *La Cellule*, **30**.

GUILLIERMOND, A. (1914). "État actuel de la question de l'évolution et du rôle physiologique des mitochondries." *Rev. Gén. Bot.* **26**.

GUILLIERMOND, A. and MANGENOT, G. (1922). "Sur la signification de l'appareil réticulaire de Golgi." *C. R. Acad. Sci.* **174.**

HARPER, R. A. (1919). "The structure of protoplasm." *Am. Journ. Bot.* **6.**

HARVEY, L. A. (1925). "On the relation of the mitochondria and Golgi apparatus to yolk-formation in the egg cells of the common earthworm, *Lumbricus terrestris.*" *Quart. Journ. Micr. Sci.* **69.**

HEIDENHAIN, M. (1900). "Die Centralkapseln und Pseudochromosomen in den Samenzellen von Proteus, etc." *Anat. Anz.* **18.**

HIRSCHLER, J. (1913). "Über die Plasmastrukturen in den Geschlechtszellen der Ascariden." *Arch. Zellf.* **9.**

—— (1914). "Ueber Plasmastrukturen (Golgischer Apparat, Mitochondria u. a.) in den Tunicaten-, Spongien- und Protozoenzellen." *Anat. Anz.* **47.**

—— (1915). "Ueber ein Verfahren zur gleichzeitigen Darstellung des Golgischen Apparates und der Mitochondrien des Zellenplasmas in differenten Farben." *Zeit. f. Wiss. Mikr. u. Tech.* **32.**

—— (1916). "Ueber die Plasmakomponenten der weiblichen Geschlechtszellen." *Arch. f. Mikr. Anat.* **89.**

—— (1918). "Ueber die Plasmakomponenten der weiblichen Geschlechtszellen." *Arch. f. Mikr. Anat.* **91.**

HOLMGREN, E. (1899). *Anatomische Hefte,* **12.**

—— (1914). "Trophospongien spinaler Ganglienzellen." *Anat. Anz.* **46.**

—— (1915). "Die Trophospongien spinaler Ganglienzellen." *Ark. f. Zoologi,* **9.**

HOVEN, H. (1910 *a*). "Sur l'histogénèse du système nerveux péripherique et sur le rôle des chondriosomes dans la neurofibrillation." *Arch. de Biol.* **25.**

—— (1910 *b*). "Contribution à l'étude du fonctionnement des cellules glandulaires. Du rôle du chondriome dans la sécrétion." *Anat. Anz.* **37.**

JOYET-LAVERGNE, M. PH. (1924). *C. R.*

JUST, E. E. (1912). "The morphology of the normal fertilisation in *Platynereis megalops.*" *Journ. Morph.* **26.**

KING, S. D. (1924). "Oogenesis in *Lithobius forficatus.*" *Scient. Proc. Roy. Dub. Soc.* **18.**

—— (1926). "Cytological observations on *Haplosporidium chitonis.*" *Quart. Journ. Micr. Sci.* **70.**

KINGSBURY, B. F. (1912). "Cytoplasmic fixation." *Anat. Rec.* **6.**

LAMS and DOORME (1908). "Nouvelles recherches sur la maturation et la fécondation, etc." *Arch. Biol.* **23.**

LEE (1921). *The microtomist's vade-mecum.*

LEWIS, M. R. and LEWIS, W. H. (1914). "Mitochondria in tissue cultures." *Science,* N.S. **39.**

—— (1915). *Am. Journ. Anat.* **17.**

LEWIS, M. R. and ROBERTSON, W. R. B. (1916). "The mitochondria and other structures observed by the tissue culture method in the male germ cells of *Chorthippus curtipennis* Scudd." *Biol. Bull.* **30.**

LEWITSKI, G. (1910). "Ueber die Chondriosomen in pflanzlichen Zellen." *Ber. deut. bot. Ges.* **28.**

—— (1914). "Die Chondriosomen als Secretbildner bei den Pilzen." *Ber. deut. bot. Ges.* **31.**

LILLIE, F. R. (1912). "Studies of fertilisation in *Nereis,* etc." *Journ. Exp. Zool.* **12.**

LOWSCHIN, A. M. (1913). "Myelinformen und Chondriosomen." *Ber. deut. bot. Ges.* **31.**

—— (1914). "Vergleichende experimental-cytologische Untersuchungen über Mitochondrien in Blättern der höheren Pflanzen." *Ber. deut. bot. Ges.* **32.**

LOYEZ, M. (1909). "Les premiers stades de la vitellogénèse chez quelques Tuniciers." *C. R. Assoc. Anat.*

LUDFORD, R. J. (1921). "Patella." *Journ. Roy. Micr. Soc.*

—— (1924). "Experiments on the impregnation of the Golgi apparatus by means of osmium tetroxide." *Journ. Roy. Micr. Soc.*

—— (1925 *a*). "Some modifications of the osmic acid methods in cytological technique." *Journ. Roy. Micr. Soc.*

—— (1925 *b*). "Cell organs during secretion in the Epididymis." *Proc. Roy. Soc. Lond.,* Aug. 1.

LUNA, E. (1913). "Sulla importanza dei condriosomi nella genesi delle miofibrille." *Arch. Zellf.* **9.**

LUNDEGARDH, H. (1910). "Ein Beitrag zur Kritik zweier Vererbungshypothesen." *Jahrb. Wiss. Bot.* **48.**

MAYER, A., RATHERY, F. and SCHAEFFER, G. (1914) "Les granulations ou mitochondries, etc." *Journ. de Physiol. et de Path. gén.* **16.**

MEVES, F. (1904). "Ueber das Vorkommen von Mitochondrien bezw. Chondriomiten in Pflanzenzellen." *Ber. deut. bot. Ges.* **22.**

—— (1907 *a*). "Die Spermatocytenbildung bei der Honigbiene." *Arch. fur Mikroskopische Anatomie,* **70.**

—— (1907 *b*). "Ueber Mitochondrien bezw. Chondriokonten in den Zellen junger Embryonen." *Anat. Anz.* **31.**

MEVES, F (1907 c). "Die Chondriokonten in ihrem Verhältnis zur Filarmasse Flemmings." *Anat. Anz.* **31.**

—— (1908). "Die Chondriosomen als Träger erblicher Anlagen. Cytologische Studien am Hühnerembryo." *Arch. Mikr. Anat.* **72.**

—— (1909). "Ueber Neubildung quergestreifter Muskelfäsern nach Beobachtungen am Hühnerembryo." *Anat. Anz.* **34.**

—— (1911). "Ueber die Beteilung der Plastochondrien an der Befruchtung des Eies von *Ascaris*." *Arch. f. Mikr. Anat.* **76.**

—— (1914 a). "Verfolgung des Mittelstückes des Echinidenspermiens, etc." *Arch. f. Mikr. Anat.* **82.**

—— (1914 b). "Was sind die Plastosomen?" *Anat. Anz.* **85.**

—— (1918). "Die Plastosomentheorie der Vererbung, etc." *Arch. f. Mikr. Anat.* **92.**

MEYER, A. (1911). "Bemerkungen zu G. Lewitski, etc." *Ber. deut. bot. Ges.* **29.**

MONTGOMERY, T. H. (1911). "The spermatogenesis of an Hemipteran, Euschistus." *Journ. Morph.* **22.**

MOORE, J. E. S. (1893). "Mammalian spermatogenesis." *Anat. Anz.* **7.**

MOREAU, F. (1914). "Le chondriosome et la division des mitochondries chez les *Vaucheria*." *Bull. Soc. Bot. France*, **61.**

MOTTIER, D. M. (1918). "Chondriosomes and the Primordia of Chloroplasts and Leucoplasts." *Ann. Bot.* **32.**

MURRAY (1919). *Canc. Res. Rep.*

NASSONOV, D. (1923). "Das Golgische Binnennetz und seine Beziehungen zu der Sekretion. Untersuchungen uber einige Amphibiendrusen." *Arch. f. Mikr. Anat.* **97.**

—— (1924). "Morphologische und experimentelle Untersuchungen an einigen Säugetierdrusen.' *Arch. f. Mikr. Anat. u. Entwick.* **100.**

NATH, V. (1924). "Oogenesis of *Lithobius forficatus*." *Proc. Camb. Phil. Soc. Biol. Sci.*

—— (1925 a). "Spermatogenesis of *Lithobius forficatus*." *Proc. Camb. Phil. Soc. Biol. Sci.*

—— (1925 b). "Cell inclusions in the oogenesis of scorpions." *Proc. Roy. Soc. Lond.* **98.**

—— (1925 c). "Mitochondria and sperm-tail formation, with particular reference to moths, scorpions and centipedes." *Quart. Journ. Micr.* **69.**

—— . "Oogenesis of *Julus terrestris*." *Quart. Journ. Micr. Sci.* (In the Press.)

NICHOLSON, N. C. (1916). "Morphological and microchemical variations in the mitochondria in the cells of the central nervous system." *Am. Journ. Anat.* **19.**

—— (1923). "A cytological study of the nature of Rickettsia in Rocky Mountain spotted fever." *Journ. Exp. M.* **37.**

PAYNE, F. (1916). "A study of the germ cells of *Gryllotalpa*." *Journ. Morph.* **28.**

PENFIELD, W. G. (1921). "The Golgi apparatus and its relationship to Holmgren's trophospongium in nerve cells. Comparison during redispersion." *Anat. Rec.* **22.**

PENSA, A. (1914). "Ancora a proposito di condriosomi e pigmento autocianico nelle cellule vegetali." *Anat. Anz.* **46.**

PORTIER, P. (1917). "Rôle physiologique des symbiotes." *C. R. Acad. Sci.* **165.**

—— (1918). "Les symbiotes." Paris, Masson et Cie.

—— (1919). "Réponse à la communication de Cl. Regaud." *C. R. Soc. Biol.* **82.**

RAU, A. S., BRAMBELL, F. W. R. and GATENBY, J. B. (1925). "Observations on the Golgi bodies in the living cell." *Proc. Roy. Soc. Lond.* Feb.

RAU, A. S. and LUDFORD, R. J. (1925). "Variations in the form of the Golgi bodies during the development of neurones." *Quart. Journ. Micr. Sci.* **69.**

REGAUD, C. (1908). "Sur les mitochondries de l'épithélium séminal." *C. R. Soc. Biol.* **65.**

—— (1909 a). "Attribution aux 'formations mitochondriales' de la fonction générale d'extraction et de fixation électives, etc." *C. R. Soc. Biol.* **66.**

—— (1909 b). "Participation des chondriomes à la formation des grains de sécrétion, etc." *C. R. Soc. Biol.* **66.**

—— (1910). "Étude sur la structure des tubes séminifères, etc." *Arch. d'Anat. Micr.* **11.**

—— (1911). "Les mitochondries, organites du protoplasma, etc." *Revue de Médecine.*

RUDOLPH, K. (1912). "Chondriosomen und chromatophoren, etc." *Ber. deut. bot. Ges.* **30.**

SAPÉHIN, A. A. (1915). "Untersuchungen uber die Individualität der Plastide." *Arch. Zellf.* **13.**

SCHERRER, A. (1914). "Untersuchungen über Bau und Vermehrung der Chromatophoren und das Vorkommen von Chondriosomen bei Anthoceros." *Flor.* **107.**

SHAFFER, E. L. (1920). *Biol. Bull.* **38.**

SHARP, L. W. (1921). *An Introduction to Cytology.*

TERNI, T. (1914). *Arch. Zellf.* **12.**

TROJAN, E. (1919). "Bakteroiden, Mitochondrien und Chromidien." *Arch. f. Mikr. Anat.* **93.**

VAN DER STRICHT, O. (1905). "Structure de l'œuf ovarique de la femme." *Bull. de l'Acad. roy. de Belgique.*

VON BAMBEKE (1898). "Recherches sur l'oocyte de *Pholcus phalangioides*." *Arch. d. Biol.* **15**.

VON DERSCHAU, M. (1914). "Zum Chromatindualismus der Pflanzenzelle." *Arch. Zellf.* **12**.

VON WINIWARTER, H. (1912). "Observations cytologiques sur les cellules interstitielles du testicule humain." *Anat. Anz.* **41**.

WALLIN, I. E. (1922). "On the nature of mitochondria." *Am. Journ. Anat.* **30**.

—— (1923 *a*). "VI. A comparative study of the fragility of bacteria and Mitochondria." *Anat. Rec.* **25**.

—— (1923 *b*). "VII. The independent growth of mitochondria in artificial culture media." *Anat. Rec.* **25**.

—— (1923 *c*). "A. The demonstration of mitochondria in tissue smears. B. Demonstration of mitochondria grown in artificial culture media." *Anat. Rec.* **25**.

—— (1923 *d*). "The Mitochondria problem." *Am. Nat.* **57**.

WEIGLE, R. (1912). "Vergleichend-zytologische Untersuchungen über den Golgi-Kopschschen Apparat, etc." *Bull. Int. Acad. Sci. Cracovie*, **57**.

WILSON, E. B. (1925). *The cell in Development and Heredity*.

GLUTATHIONE

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GLUTATHIONE is a dipeptide of cystine and glutaminic acid isolated by Hopkins (1921) from yeast, mammalian muscle and liver. Its constitution was determined by Quastel, Stewart and Tunnicliffe (1923) to be that of diglutaminyl cystine.

$$
\begin{array}{llll}
CH_2\!-\!S\!-\!S\, -\, CH_2 & & & \\
| & & | & \\
CHNH\!-\!CO & CHNH\!-\!CO & & \\
| & | & | & | \\
COOH & CH_2 & COOH & CH_2 \\
& | & & | \\
& CH_2 & & CH_2 \\
& | & & | \\
& CHNH_2 & & CHNH_2 \\
& | & & | \\
& COOH & & COOH.
\end{array}
$$
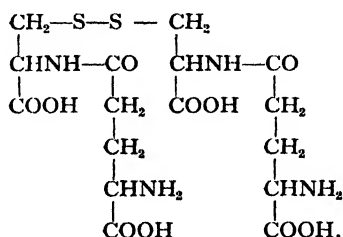
Like the amino acid cystine, glutathione can be obtained in two forms—the disulphide and the sulphydryl form, which will be referred to as G_2S_2 and G.SH respectively.

The structure assigned to glutathione was finally confirmed by the synthesis of the compound given above and the demonstration of the identity of the natural and synthetic products (Stewart and Tunnicliffe, 1925).

From 50 kg. of yeast 20 gm. of the pure substance may be obtained, *i.e.* 0·04 per cent. (Hopkins, 1925). This figure is much below that obtained by direct estimation of the —SH groups in the yeast extract (0·15 per cent. (Tunnicliffe, 1925)), but large losses in the isolation are certain to occur.

A table is given showing some typical values for various tissues:

	Estimated %	Isolated %
Skeletal muscle, Rat	0·034	0·017
Yeast	0·15–0·2	0·04
Liver, Rabbit	0·22–0·35	—

Holden (1925) has demonstrated the presence of glutathione in the blood corpuscles of sheep, goats, rabbits and rats and isolated about 0·05 gm. per litre from sheep's blood.

In the tissues glutathione is apparently present almost entirely in the reduced form (Tunnicliffe, 1925; Holden, 1925), a result which is not surprising when it is realised that the tissues can rapidly reduce added amounts of G_2S_2 to the sulphydryl form.

The reduced form of course is responsible for the vivid coloration given by tissues or tissue extracts with sodium nitroprusside and ammonia; this reaction was obtained by Hopkins (1921) from the following tissues or extracts as types:

Muscle and other tissues of the earthworm; adductor muscle of the oyster; muscles of the blowfly; muscles and other organs of the lobster; muscles of skate and cod—other organs of fish not examined; all organs of the frog and all organs of every mammal examined.

Generally the reaction was found to be less intense in cold-blooded animals than in warm.

It is of course possible that in certain cases the reaction is due to a different if analogous substance from glutathione; so far, however, no compound of such a type has been isolated. Abderhalden and Wertheimer (1923) have indeed suggested that free cysteine may exist in the tissues in amounts comparable with that of the dipeptide; Hopkins (1925) states that the concentration of free cysteine is probably very much less than 1/20 of that of the dipeptide.

The physiological relations between glutathione and the tissues may now be considered.

Fresh tissue, or tissue which has been thoroughly washed with distilled water, is able rapidly to reduce the disulphide form to the sulphydryl type

$$G_2S_2 \xrightarrow{2H} 2G.SH.$$
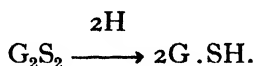
Fresh tissues reduce methylene blue, as does the dipeptide in its sulphydryl form. Tissues which have been thoroughly washed, however, reduce methylene blue with extreme slowness; and the disulphide form of glutathione has no action whatever on methylene blue.

When oxidised glutathione is added to a tissue preparation in the presence of methylene blue in a buffer solution of pH 7·4 a reducing system is set up and the methylene blue is rapidly reduced to its leuco base.

Thus for a washed muscle preparation Hopkins (1921) obtained the following results:

	Methylene blue	Oxidised glutathione	pH	Reduction time
Muscle (washed), 0·2 gm.	0·3 c.c.	0 mg.	7·4	3 hr. +
,, ,, 0·2 ,,	0·3 ,,	8 ,,	7·4	0 hr. 10 min.

The reactions occurring in the system so constituted may be expressed:

(i) $T{<}^H_H + G{-}S{-}S{-}G \longrightarrow T + 2G.SH,$

(ii) $2G.SH + M.B \longrightarrow G{-}S{-}S{-}G + M.BH_2,$
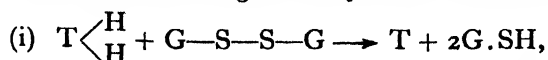
where $T{<}^H_H$ represents the hydrogen donator of the tissue preparation,

and M.B and M.BH$_2$ methylene blue and leuco methylene blue respectively.

The two reactions in series occur rapidly, but the direct reaction between the tissue and methylene blue occurs only with great slowness.

Hopkins and Dixon (1922) later made the important observation that the system described above was thermostable and was thus sharply differentiated from ordinary enzymic systems capable of reducing methylene blue (Thunberg, 1920). By boiling washed muscle in water, washing with alcohol and drying *in vacuo*, they obtained a dry powder which they termed the thermostable residue.

They showed that the maximum amount of methylene blue such a preparation could reduce was independent of the amount of G_2S_2 added to the system.

Tunnicliffe (1925) measured directly the maximum amount of the disulphide which a given weight of such a preparation would reduce, and found this to correspond extremely well with the maximum reduction which occurred with methylene blue.

There is therefore little doubt that the dipeptide plays the reversible part indicated in the equation below:

$$G—S—S—G \rightleftharpoons 2G—SH.$$

Hopkins and Dixon (*loc. cit.*) extended the observations with methylene blue to the system where oxygen is the final oxidising agent. The facts correspond generally with those ascertained for methylene blue. The thermostable preparation alone shows only a small and very slow oxygen uptake. With the addition of G_2S_2 in $M/200$ solution approximately the oxygen uptake at pH 7·6 is brisk and of the order of 400 c.mm. per gm. of dry powder, using Clark and Lub's phosphate buffer solution.

They found (as with methylene blue) that when the dipeptide forms an oxidising system with washed muscle, it suffers itself no change except the reversible one involving the sulphur group alone.

Hopkins (1925) afterwards found that in Ringer's solution at pH 7·6 the oxygen uptake measured in the same way was of the order of 2000 c.mm./gm., and again demonstrated that the total uptake was independent of the amount of G_2S_2 added: the velocity of course varied with the concentration.

Tunnicliffe (1925) found that there was no significant difference in the amount of G_2S_2 which could be reduced by a given weight of muscle powder in phosphate buffer solution or in Ringer's solution.

Meyerhof (1923) using thioglycollic acid as a sulphur compound made observations similar to those of Hopkins and Dixon.

Before proceeding to a further consideration of the functions of glutathione in oxidising systems of the type described, attention must be directed to the work of Warburg and Sakuma (1923). They showed that the —SH group of thiol compounds is not strictly autoxidisable but is oxidised by atmospheric oxygen only in the presence of minute traces of iron. Harrison (1924) confirmed this in the case of glutathione.

As pointed out by Hopkins (*loc. cit.*) the laborious nature of the process of removal of the traces of iron precludes the possibility of controlling all the tissue

experimental work with iron-free glutathione: in any case little difference was found using iron-free preparations from those obtained before Warburg's observation. The metal is only another link in the chain, and does not remove any significance from the results to be described as regards the functions of the —SH groups of glutathione in respiration.

It will be convenient at this point to consider the results obtained by a study of the influence of glutathione on the oxidation of fatty acids and fats, in order to appreciate their significance in the study of the nature of the tissue hydrogen donators.

In aqueous systems at pH 3·0–4·0 containing G.SH and an emulsion of an unsaturated fatty acid (linoleic; linolenic) the following changes occur on aeration of the system. The concentration of the —SH group is long maintained as would be expected from the results of Dixon and Tunnicliffe (1923). The oxygen uptake of the system is much in excess of that necessary for the conversion of the —SH groups to the —S—S— form; an amount of fatty acid corresponding to this excess uptake is oxidised.

With alteration of the hydrogen ion concentration to the neutral point or slightly on the alkaline side (pH 7·4–7·6) the relations are decidedly altered. The —SH group is rapidly oxidised and only small quantities of fat are oxidised before the system becomes inert. Oxidised glutathione G_2S_2 has of course no effect on unsaturated fatty acids.

The following table quoted from Hopkins (1925) will clearly show the difference in the two cases. It will be noticed that at pH 7·4–7·6 the oxygen uptake of the fatty acid is approximately equal to the oxygen equivalent of the SH group. The latter may be calculated from the equation

$$4G.SH + O_2 \longrightarrow 2G_2S_2 + 2H_2O$$
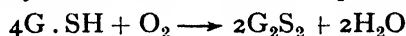
or obtained experimentally using an equal amount of G.SII alone.

No.	Material 10 mg.	pH	Time for uptake hrs.	Total uptake mm.³	O_2 equivalent of —SH mm.³	O_2 to fat mm.³	Remarks
1	Linolenic acid	3·5	6	210	32	178	Reaction still progressing
2	Linoleic acid	3·5	6	140	32	108	
3	Mixed acids from linseed oil	3·2	5½	710	200	510	
4	Mixed acids	7·6	3	325	165	160	Uptake complete
5	Mixed acids	7·6	4	180	90	90	
6	Linoleic acid	7·4	5	250	132	118	

The behaviour of systems of unsaturated fatty acid + G.SH is thus essentially identical with that described by Meyerhof (1923) for the systems linolenic acid—thioglycollic acid and lecithin—thioglycollic acid.

So far as the systems at a pH of 3·0–4·0 are concerned, the views advanced by Meyerhof as to the mechanism of the reaction seem to be in accord with the experimental evidence.

He assumes that two —SH groups first form a peroxide which then decomposes in such a way as to transfer the whole of the oxygen to the unsaturated linkages of the fatty acids, with reappearance of the original —SH groups. Here the —SH compound functions as a catalyst and is concerned chiefly with oxygen transport; the reaction falls into the type summarised by Engler as

$$A + O_2 \longrightarrow AO_2$$
$$AO_2 + 2B \longrightarrow A + 2BO,$$
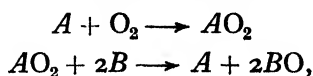
where A is the autoxidisable substance (the —SH group) and B the acceptor (the unsaturated fatty acid).

In neutral or faintly alkaline solution the reaction falls apparently into the class of induced or coupled reactions of the type

$$A + O_2 \longrightarrow AO_2$$
$$AO_2 + B \longrightarrow AO + BO,$$
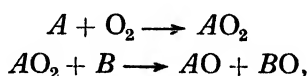
where the induced oxidation will finish with the complete conversion of $A \longrightarrow AO$.

The results obtained when the glycerides of the unsaturated fatty acids are used differ considerably from those obtained with the free acids.

At pH 3–4 there is a long induction period during which the system is almost inert (a phenomenon never observed with the free acids); at pH 7·4–7·6 the equi-partition of oxygen previously described for the free acids is not seen. There is no induction period, but a rapid uptake of oxygen until almost the whole of the G.SH has been oxidised. The rate of uptake then falls off and proceeds linearly until the fat is almost completely oxidised. During this period the concentration of —SH, as shown by the nitroprusside test, is very small. The explanation of the difference in behaviour of free fatty acids and their glycerides is by no means clear; it is, how-ever, of importance in that the reactions described (pH 7·4–7·6) take place at about the pH of animal tissues. Lecithin behaves like the free acids, rather than the glycerides.

The work of Hopkins (1925) on lipoid-free tissue preparations prepared by thorough extraction with alcohol and ether has led to most interesting results.

At pH 3–4 such a preparation from muscle shows no oxygen uptake on addition of G_2S_2; at pH 7·6 the uptake is one-third or more of that of an unextracted preparation.

It is clear that the material oxidised is not a lipoid; and the fact that the extracted tissue possesses intact its power of reducing G_2S_2 to G.SH is in accord with the observation that linolenic acid and lecithin have no power to accomplish this change (Tunnicliffe, 1925).

The oxidation of such fat-free preparations in contact with G_2S_2 depends on the presence in them of what is termed by Hopkins and Dixon (1922) a fixed —SH group. It should be noted that particularly as regards muscle, a strong nitro-prusside reaction remains after the whole of the soluble glutathione has been removed by thorough washing.

This fixed —SH group is very resistant to oxidation by molecular oxygen; but

by treatment of the muscle tissue with G_2S_2 aerobically or anaerobically, the tissue loses its nitroprusside reaction. Anaerobically the reaction appears in the fluid owing to the presence of G.SH in solution as a result of the reduction of G_2S_2 by the tissue preparation.

If a tissue which has no observable nitroprusside reaction, following treatment as described above, be treated with solutions of a thiol compound (thioglycollic acid, cysteine, or reduced glutathione) of sufficient concentration, the reaction is again displayed with intensity undiminished.

Like that in the original tissue, the restored group cannot be removed by washing, is resistant to direct oxidation, but disappears rapidly on treatment with G_2S_2. By repeating the treatment with a thiol solution it may again be restored further.

As a result of the disappearance of the original fixed —SH group a muscle preparation becomes incapable of taking up oxygen in the presence of G_2S_2, but after restoration it is capable of taking up an amount of oxygen greatly in excess of the oxygen equivalent of the —SH restored.

The evidence brought forward by Hopkins (*loc. cit.*) makes it clear that the disappearance and reappearance of the fixed —SH group are not due to interchange between molecules adsorbed on the tissue and molecules in solution, but represent a true oxidation and reduction.

It seems clear that the fixed —SH group is the only reducing agency in the thermostable residue (or even in washed muscle) which reduces glutathione.

The following results were obtained by Hopkins for a thermostable preparation of rabbit's muscle:

Treatment of material before determination of uptake	Total O_2 uptake when shaken in G_2S_2 sol. ($M/200$) at pH 7·6; c.mm. per gm.
Extracted (alcohol-ether-alcohol)	1020
Oxidised aerobically with G_2S_2 till free from nitroprusside reaction	0
Reduced in G.SH solution ($M/10$)	1850
Oxidised anaerobically with G_2S_2	—
Reduced in cysteine solution ($M/10$)	—
Oxidised aerobically and then reduced by thioglycollic acid ($M/10$)	2010

It appears that the properties of the fat-free muscle residue described above are due to the properties of its proteins and that the oxidations described apparently involve no other material. Certain proteins give a nitroprusside reaction or can be made to give one by treatment such as denaturation. Thus Arnold (1914) showed that muscle proteins gave an intense reaction; whereas the proteins of blood plasma gave no reaction. On denaturation, however, serum globulin and egg albumin acquire this power (Heffter, 1907).

More powerful is the result of exposing certain pure proteins as coagula to the action of concentrated solutions of thiol compounds. The nitroprusside reaction

exhibited by the protein after this treatment is much more intense than that produced by denaturation. In the case of the serum proteins this is marked, for by heat denaturation they develop no reaction whatever, but with a thiol solution acquire a very marked reaction.

Different proteins vary considerably in the amount of —SH group established in them by this treatment; thus for the following proteins the oxygen equivalent of the —SH group so established was found to be:

	Oxygen equivalent of —SH group established
Mixed serum proteins coagulated and treated with $M/10$ G.SH	300 mm.^{3} per gm.
Crystalline egg albumin	160 mm.^{3} ,,
Crude fibrin	<60 mm.^{3} ,,
Gelatin	Incapable of developing any —SH group

Those proteins which fail to exhibit a nitroprusside reaction show no power to reduce solutions of G_2S_2. The actual oxygen uptake of the proteins having this —SH group, at pH 7·6, in the presence of G_2S_2 is greatly in excess of the oxygen equivalent of the —SH group.

Thus the mixed serum proteins referred to gave an oxygen uptake of 2350 mm.^{3} per gm. and as in the case of the fat-free thermostable preparations of muscle the oxidation stopped because of the disappearance of the fixed —SH group.

By treating anew with a concentrated thiol compound, a further uptake is obtained, and so on. The final uptake reaches at least 10 c.c. of oxygen per gm. of serum protein and possibly much more. The protein displayed no oxygen uptake alone, and none with G_2S_2 at pH 3·5–4·5.

It is important to note that before certain proteins can be "reduced" by contact with thiol solutions they must first be denatured or coagulated. This is not the case, however, with the insoluble proteins of muscle which can undergo this alternate reduction and oxidation in their native state.

So far the facts are insufficient to decide whether the oxidation of protein conforms to either of the two types of reaction mentioned in connection with the oxidation of the unsaturated fatty acids, or whether it is a process best considered as one of hydrogen activation and transport.

Tunnicliffe (1925) showed that the reduction of G_2S_2 by a thermostable preparation of muscle is carried on over a wide range of pH, and is much less affected by pH than the oxidation of G.SH to G_2S_2 (Dixon and Tunnicliffe, 1923). The same holds for a "reduced" protein.

At pH 3·5–4·5 the oxidation does not proceed since the system is stable; if fat, however, is present, oxidation of the fat proceeds on the lines already indicated, the G.SH acting as a catalyst.

At pH 7·6 the oxidation of the G.SH proceeds rapidly compared to the rate of

reduction of G_2S_2 by the fixed —SH group of the protein; consequently the G.SH concentration never reaches more than a very small value. The oxidation of the protein proceeds, and together with it the oxidation of fat, to the certain limited extent already indicated.

At an intermediate pH (6·5–6·8) the oxidation of the protein still goes on, but the concentration of G.SH is higher than at pH 7·6 owing to the slower rate of the reaction

$$2G.SH \longrightarrow G.S.S.G.$$
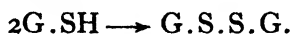
It would seem then that here glutathione is acting as an intermediate hydrogen acceptor; a view which is supported by the fact that when a muscle preparation or a reduced protein reacts anaerobically with G_2S_2 the maximal reduction of the latter corresponds quantitatively with the reducing capacity of the fixed —SH group (Tunnicliffe, *loc. cit.*; Hopkins, *loc. cit.*). Aerobically, however, the oxygen uptake is ten times the oxygen equivalent of the fixed —SH group.

Hopkins suggests tentatively that the explanation of the anomaly may lie in the fact that the fixed sulphur group may be an additional hydrogen acceptor. There may be factors in the protein which although ultimately oxidisable are unable to reduce G_2S_2, but can reduce the fixed —SH group again after this has reacted with glutathione.

It is probable that the identification of the groups concerned in this additional oxygen uptake may be accomplished by examination of a protein which has been alternately reduced and oxidised a sufficient number of times to alter distinctly its chemical properties. The results of this examination may well indicate the additional factors concerned. One thing is certain; that whether the fixed —SH group is itself the primary hydrogen donator or not, the key move of the various oxidations described is, so far as our knowledge goes, the production of an —SH group in solution by the slow reducing action of the fixed —SH group on oxidised glutathione. What course the oxidation will then take. after the establishment of this soluble G.SH will depend on the hydrogen ion concentration of the system; and to a certain extent on the relative amounts of fat and protein present. So far the evidence obtained from a study of these oxidations in muscle is concerned entirely with protein and fat. The work of Miss Thurlow (1925) must, however, be borne in mind in this connection.

The oxidation of G.SH by atmospheric oxygen, it has been assumed, occurs with formation of a peroxide, either organic or hydrogen peroxide. When this oxidation occurs in the presence of a peroxidase, Miss Thurlow showed that an oxidation of other substances (*e.g.* nitrite) is induced which does not take place in the absence of the peroxidase.

It is possible that along these lines the functions of glutathione may be brought into relation with the carbohydrate metabolism in muscle and other tissues. For a further knowledge of the functions of glutathione progress would seem to depend on the elucidation of the following points:

(*a*) The nature of the molecule to which the fixed —SH group is linked. In this connection the suggestions put forward by Harris (1923) to account for the

appearance of a nitroprusside reaction in egg albumin after denaturation may be of value.

(b) A determination of the particular groups in the protein molecule which are oxidised aerobically in the presence of G_2S_2 and account for the oxygen uptake of ten times the oxygen equivalent of the fixed —SH group. Are the amino groups of the constituent amino acids involved in the oxidation; or is it merely another example of an oxidation of unsaturated linkages catalysed by a thiol group?

(c) A further study of the catalytic oxidation of the unsaturated fatty acids and glycerides. Hopkins (loc. cit. note, p. 798) makes it clear that the study of linseed oil should be repeated on purified individual glycerides; and it would probably be of interest to correlate the effect of glutathione on them with the previous history and treatment of the unsaturated acid or fat.

Although the results described have been obtained under artificial conditions, they are not on that account to be regarded as divorced from events actually occurring in the living cell. They are given by actual cell constituents, in many cases interacting in media of hydrogen ion concentrations approaching that of the living organism, so that it is difficult to conceive that a mere alteration in the conditions under which they react can in any way detract from their reality and importance.

The relation of the glutathione system to other oxidation-reduction processes may now be briefly considered.

In the anaerobic oxidations which are brought about by animal tissues, the simultaneous presence of a reducible substance—a hydrogen acceptor—is necessary. The means by which these oxidations are accomplished are generally considered from two points of view, those of Bach (1912) and of Wieland (1914).

Bach imagines a splitting of water molecules

$$R + \begin{matrix} OH\ H \\ OH\ H \end{matrix} \ +\ A \ \longrightarrow \ R\begin{matrix} OH \\ OH \end{matrix} + A\begin{matrix} H \\ H \end{matrix}$$

Oxygen acceptor   Hydrogen acceptor $\downarrow$

$$RO + H_2O$$
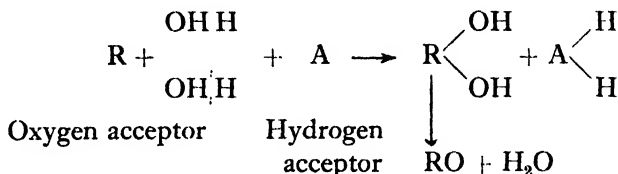
whereas Wieland assumes that there is an activation of hydrogen

$$R\begin{matrix} H \\ H \end{matrix} + A \longrightarrow R + A\begin{matrix} H \\ H \end{matrix}$$
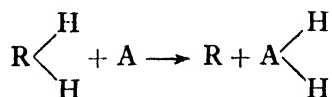
If the substance R be assumed to react in the form of its hydrate Wieland's scheme becomes

$$R \cdot O\begin{matrix} H \\ H \end{matrix} + A \longrightarrow RO + A\begin{matrix} H \\ H \end{matrix}$$
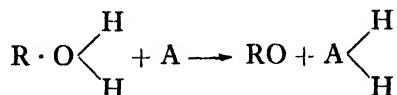
a result very similar to that of Bach.

The Schardinger enzyme of milk furnishes a good example of this type of process. Milk alone will not reduce methylene blue, but if a little aldehyde be added the methylene blue is reduced and the aldehyde simultaneously oxidised.

On Bach's view we have

$$RCHO + O\!\!<_{H}^{H} + MB \longrightarrow RCOOH + MBH_2$$
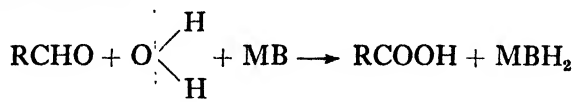
whilst Wieland would regard the process as

$$R \cdot \overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle OH}{|}}{C}}\!\!-\!OH + MB \longrightarrow RCOOH + MBH_2$$
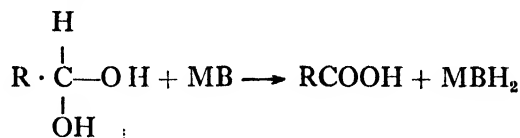
the aldehyde acting in its hydrated form.

Milk also contains an enzyme which oxidises xanthin or hypoxanthin to uric acid if a hydrogen acceptor, methylene blue or sodium nitrite, is present.

In aerobic oxidations it is probable that oxygen becomes the hydrogen acceptor and is converted into hydrogen peroxide

$$R\!\!<_{\displaystyle H}^{\displaystyle H} + \underset{\displaystyle O}{\overset{\displaystyle O}{\|}} \longrightarrow R + H_2O_2$$

$$RO\!\!<_{\displaystyle H}^{\displaystyle H} + \underset{\displaystyle O}{\overset{\displaystyle O}{\|}} \longrightarrow RO + H_2O_2$$
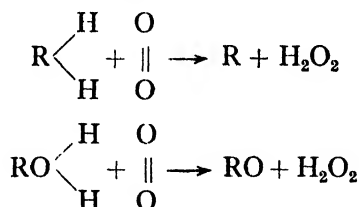
Evidence is accumulating that in aerobic oxidations such as the aerobic oxidation of xanthin to uric acid it is hydrogen peroxide which is formed and not an organic peroxide as was formerly supposed.

It will be noticed that following Wieland's views, the conception of a reductase, an oxidase or a mutase as entirely different classes of enzymes is no longer necessary; they are merely special cases of the more general phenomenon. Thus the Schardinger enzyme plus an aldehyde would be regarded as a reductase.

Glutathione fits in well with Wieland's views on the activation of hydrogen and may be regarded as pre-eminently an intermediate hydrogen acceptor. When the reduced form of the dipeptide is undergoing oxidation by atmospheric oxygen, hydrogen peroxide is produced and this peroxide may bring about a direct oxidation of other materials, or an indirect oxidation of a different type through the presence of the enzyme peroxidase, as was previously pointed out[1]. In this case the system may be regarded as one of oxygen transport.

So far as the evidence is available, glutathione plays no direct part in any of the known enzyme systems: its function in brief is that of a chemical catalyst, a rôle for which its properties make it admirably suited.

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[1] The function of catalase would thus appear to be to prevent the accumulation of harmful amounts of hydrogen peroxide in the cell.

BIBLIOGRAPHY.

ABDERHALDEN and WERTHEIMER (1923). *Pflüger's Arch.* **200**, 76.
ARNOLD (1914). *Z. physiol. Chem.* **70**, 300.
BACH (1912). *Biochem. Zeitschr.* **38**, 154.
DIXON and TUNNICLIFFE (1923). *Proc. Roy. Soc. London*, B, **94**, 266.
HARRIS (1923). *Proc. Roy. Soc. London*, B, **94**, 428.
HARRISON (1924). *Bioch. Journ.* **18**, 1009.
HEFFTER (1907). *Mediz.-Naturwiss. Arch.* **1**, 81–104 (*Chem. Zentr.* **11**, 822).
HOLDEN (1925). *Bioch. Journ.* **19**, 727.
HOPKINS (1921). *Bioch. Journ.* **15**, 286.
—— (1925). *Bioch. Journ.* **19**, 787.
HOPKINS and DIXON (1922). *Journ. Biol. Chem.* **54**, 529.
MEYERHOF (1923). *Pflüger's Arch.* **199**, 531.
QUASTEL, STEWART and TUNNICLIFFE (1923). *Bioch. Journ.* **18**, 586.
STEWART and TUNNICLIFFE (1925). *Bioch. Journ.* **19**, 207.
THUNBERG (1920). *Skand. Arch. Physiol.* **40**, 1.
THURLOW (1925). *Bioch. Journ.* **19**, 175.
TUNNICLIFFE (1925). *Bioch. Journ.* **19**, 194.
—— (1925). *Bioch. Journ.* **19**, 199.
WARBURG and SAKUMA (1923). *Pflüger's Arch.* **200**, 203.
WIELAND (1914). *Ber.* **47**, 2085.

LES THÉORIES DE LA POLARITÉ DANS LES PHÉNOMÈNES DE RÉGÉNÉRATION

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(Avec six figures dans le texte.)

(Reçu le 15 juin, 1926.)

SOMMAIRE.

INTRODUCTION: LES MANIFESTATIONS DE LA POLARITÉ.

Les phénomènes de régénération chez les organismes ont fait l'objet d'innombrables publications, et, cependant, le problème de la régénération semble être encore un des plus obscurs de la Biologie générale. Actuellement, comme le dit si justement J. Loeb (1926) dans son dernier livre: "Ce dont nous avons besoin dans ce domaine, ce ne sont pas de nouveaux faits mais une méthode et un principe qui nous permettent de passer de la période d'empirisme aveugle à celle de la recherche orientée."

On trouvera dans cet article un examen comparatif et critique des principales théories qui ont été émises sur la régénération chez les Invertébrés inférieurs: la régénération se présente chez ces animaux (Coelentérés, Turbellariés, Annélides) avec un caractère de polarité linéaire qui lui donne un aspect plus schématique qu'ailleurs. D'éminents biologistes, comme T. H. Morgan, comme J. Loeb, ont

éclairé la voie, et, dans ces dernières années, C. M. Child a proposé pour l'interprétation de ces phénomènes une théorie très originale, et qui ouvre largement le champ à l'expérimentation.

Nous devons d'abord donner un aperçu des faits positifs qui sont généralement englobés sous le terme vague, d'apparence un peu métaphysique, de "polarité[1]." Ces faits peuvent être classés en deux catégories: les faits d'ordre qualitatif, les faits d'ordre quantitatif, correspondant à des phénomènes susceptibles de mesure. J'insisterai surtout sur ces résultats quantitatifs, moins généralement connus, qui correspondent à une étude plus précise des phénomènes, et servent de base aux théories récentes.

A. MANIFESTATIONS QUALITATIVES.

Des fragments tels que *abcd* (Fig. 1) du corps d'une Planaire, compris entre deux sections transversales, régénèrent normalement une tête à leur extrémité orale *ab*, une queue à leur extrémité aborale *cd*. Tel est le fait fondamental par lequel se manifeste ce qu'on a appelé depuis Allman (1864) la "polarité" de l'organisme primitif.

On a souvent comparé l'expérience précédente à celle de l'aimant brisé. Dans un cas comme dans l'autre, le sort ultérieur d'une surface telle que *cd* semble dépendre uniquement de sa position dans le fragment, et non de sa position dans l'ensemble primitif: les cellules de la section *cd* peuvent être le point de départ de la régénération d'une tête ou d'une queue, suivant qu'elles font partie du fragment *cdef* ou du fragment *abcd*.

La polarité organique se manifeste donc tout d'abord qualitativement, par la nature du régénérat (structure antérieure ou structure postérieure) dans une direction donnée. A ce point de vue, la polarité s'oppose radicalement à l'"hétéromorphose" ou renversement de la polarité, qui consiste en la régénération d'une structure antérieure au lieu et place d'une structure postérieure, ou inversement.
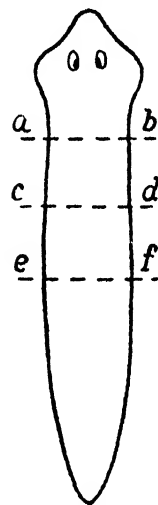
Fig. 1.

B. MANIFESTATIONS QUANTITATIVES.

Mais la polarité se manifeste aussi, à certains points de vue, par des phénomènes quantitatifs, susceptibles de mesure, et plus accessibles par là à l'expérimentation.

1°. *Degrés dans la régénération.*

La qualité même de la régénération est susceptible de degrés. La régénération antérieure de fragments de *Planaria dorotocephala* peut donner naissance, suivant Child (1911, etc.), non seulement à des extrémités antérieures normales, mais à toute une série de formations tératologiques, de têtes plus ou moins anormales (formes tératophtalmique, tératomorphique, anophtalmique), qui constituent des intermédiaires entre la régénération d'une tête normale et la non-régénération de

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[1] Nous avons choisi ce mot comme titre de cet article car, seul il englobe l'ensemble des faits dont il sera question ici. La notion de polarité, quoique vague, domine d'ailleurs, en ce qui concerne les animaux inférieurs, la plupart des faits du domaine de la régénération.

la tête (forme acéphale). La proportion de têtes normales par rapport aux têtes tératologiques, dans un lot de Planaires soumises à l'expérimentation—ce que Child a appelé "fréquence de la tête"—mesure quantitativement la régénération dans les conditions de l'expérience. Cette fréquence de la tête varie, comme on le verra plus loin, avec la taille des fragments, la région du corps dont ils proviennent, enfin avec les conditions extérieures.

Ainsi, il semble y avoir une série continue, reliant la régénération polaire typique à l'absence de régénération et à l'hétéromorphose, qui constitueraient autant de degrés de la polarité.

On peut rapprocher de ces faits des observations déjà anciennes faites sur la Tubulaire. Presque tous les expérimentateurs (Bickford, 1894; Driesch, 1897, 1899; Morgan, 1901; Child, 1907 *c*) ont constaté que, dans la régénération de fragments très courts du tronc, on obtenait, soit des polypes complets, mais de taille réduite, avec un petit morceau de tronc, soit des polypes de taille normale, mais incomplets du côté proximal (réduits, par exemple, au péristome et à la rangée distale de tentacules). Or, les formations de la première catégorie (polypes complets, mais réduits) se rencontrent plus fréquemment avec les petits morceaux prélevés dans les régions proximales du tronc primitif, tandis que les morceaux provenant des régions distales (les plus voisines du polype primitif) donnent une plus forte proportion de formes incomplètes. On peut résumer ces faits en disant, avec Driesch et Morgan, que la "tendance" à former un polype de taille normale est plus prononcée dans les régions proximales.

Enfin, chez la Tubulaire encore, suivant Driesch (1899) et Child (1907 *b*), les dimensions relatives des ébauches des deux rangées de tentacules, de même que les dimensions et les proportions du polype achevé, présentent des variations polaires, avec le niveau de la section dans le tronc primitif.

2⁰. *Vitesse de régénération et niveau de section.*

L'étude de la vitesse de régénération permet également une analyse de la polarité conduisant à des résultats quantitatifs. T. H. Morgan (1905) comprit le premier l'intérêt de ce point de vue, à la suite d'expériences sur la Tubulaire montrant que la vitesse de régénération d'un polype est d'autant plus grande que la section est plus apicale, plus proche du polype primitif.

Déjà Child (1903) avait montré que chez le Cérianthe la vitesse de la régénération et la quantité de tissu régénéré décroissent du pôle oral au pôle aboral.

Cette loi semble être générale pour la régénération du polype des Hydraires: elle s'applique à *Tubularia*, à *Obelia* (Billard, 1904; Lund, 1923), à *Pennaria* (Gast et Godlewski, 1903), à *Eudendrium* (Goldfarb, 1907), à *Corymorpha* (Torrey, 1910).

La même loi s'applique (Child, 1906 *a*) à la régénération de la tête des Planaires: la vitesse de formation de la tête, dans des fragments de *Planaria maculata* de taille constante, varie suivant la région du corps dont ils proviennent; elle est plus grande dans les régions antérieures.

Des faits analogues se retrouvent dans la régénération d'une extrémité postérieure. D'après Morgan, la régénération de la queue du Lombric est d'autant

plus rapide que la section est plus antérieure. Cette loi s'applique à la régénération d'organes ou d'appendices nombreux et variés: la vitesse de régénération est d'autant plus grande que la section est plus proximale, c'est-à-dire qu'une plus grande partie de l'organe a été excisée. Ainsi se comportent: l'extrémité caudale des Annélides *Allolobophora foetida* (Morgan, 1906), *Lumbriculus* (S. Morgulis, 1907), *Podarke obscura* (Morgulis, 1909); les bras des Astéries (King, 1900), la nageoire caudale des Téléostéens (Morgan, 1906), la queue des Amphibiens: Salamandre (Morgan, 1906), Têtard (Ellis, 1909)[1], Amblystome (Zeleny, 1917). De même, d'après Stockard (1907), la régénération du disque d'une méduse, *Cassiopea xamachana*, se fait avec une vitesse d'autant plus grande que la section est plus proche du centre; et, cela, que la régénération progresse dans le sens centripète ou dans le sens centrifuge.

Ces différences dans la vitesse de régénération avec le niveau de la section sont, comme l'ont établi Morgan (1906) pour le Lombric et Morgulis (1907, 1909) pour *Podarke* et *Lumbriculus*, indépendantes de l'état général de nutrition de l'organisme. Elles indiquent donc bien des propriétés particulières des différents niveaux, et, en ce sens, on doit les classer parmi les manifestations quantitatives de la polarité dans les organismes considérés.

C. EXTENSION DES PHÉNOMÈNES DE POLARITÉ.

1°. *La polarité chez les animaux.*

La polarité dans la régénération se présente avec le plus de netteté chez les organismes qui entrent dans la définition de ce que Driesch a appelé les "systèmes harmoniques équipotentiels," c'est-à-dire les êtres tels qu'une partie quelconque de l'organisme est capable de régénérer l'organisme entier. Tels sont les Hydraires (Hydre, Tubulaire, etc.), les Actinies, le Cérianthe, les Planaires, etc.

Tous ces êtres répondent à la définition de la polarité qualitative donnée plus haut: une même section peut régénérer, soit une structure antérieure, soit une structure postérieure, suivant qu'elle constitue l'extrémité antérieure ou l'extrémité postérieure d'un fragment.

Mais la considération des phénomènes quantitatifs nous conduit à ranger parmi les systèmes polarisés des organismes qui, comme le Lombric[2], par exemple, ne répondent pas à cette définition. L'étude des variations de la vitesse de régénération avec le niveau de la section montre en effet des phénomènes de même nature dans les deux cas, et il y a intérêt à ne pas séparer, comme semble vouloir le faire Morgan (1904), ces diverses manifestations des mêmes phénomènes.

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[1] Les recherches de Durbin (1909) et de Ellis (1909) ont montré que la vitesse de régénération de la queue des têtards varie au cours des différents stades de la régénération, mais qu'elle est cependant toujours plus grande aux niveaux antérieurs qu'aux niveaux postérieurs.

[2] Si l'on coupe un Lombric par une section transversale passant postérieurement au 15e segment, le fragment antérieur régénère une queue, mais le fragment postérieur ne régénère pas, ou régénère une queue hétéromorphique. Ainsi, une tranche de section ne peut donner naissance qu'à une queue, quelles que soient ses relations.

De même, la queue des Amphibiens, des Poissons, les bras des Echinodermes, les tentacules des Actinies, sont facilement régénérés par le fragment proximal, attenant au reste de l'organisme, tandis que le fragment distal ne régénère rien.

2°. *La polarité chez les végétaux.*

La régénération de bourgeons et de racines chez les végétaux se présente avec un remarquable caractère de polarité. Pourtant—ainsi qu'on le verra plus loin—la polarité des végétaux semble avoir des caractères différents de la polarité animale. En particulier, les variations quantitatives de la polarité animale, avec la position des fragments dans l'individu primitif, n'existeraient pas chez les végétaux, où l'individualité elle-même est très peu marquée.

3°. *Polarité et conditions externes.*

Dans un certain nombre de cas, signalés par J. Loeb (1905, 1906, 1916) et devenus classiques, les conditions extérieures ont une influence prépondérante sur la qualité de la régénération et la polarité ne se manifeste pas.

Ainsi, chez *Tubularia*, le contact avec une surface solide détermine la régénération d'un stolon, au lieu d'un polype. Il en serait de même pour d'autres Hydraires, *Pennaria*, *Margelis*.

La régénération des branches ou des stolons de l'Hydraire *Antennularia* serait déterminée principalement par l'action de la pesanteur.

On doit penser que les phénomènes internes qui se manifestent par des faits de polarité dans des Hydraires voisins, ou, chez les mêmes animaux, dans d'autres conditions, existent également chez les formes précédentes. Mais ces phénomènes de polarité doivent être d'une nature telle qu'ils peuvent être influencés par les conditions extérieures, et que cette influence puisse être prédominante dans certains cas.

D. Définition et nature de la polarité.

Les faits qui viennent d'être exposés constituent la véritable définition de la notion de polarité[1]. Ces faits relèvent-ils, dans tous les cas, d'un même mécanisme, qui justifierait leur groupement, et ferait du terme "polarité" autre chose qu'une dangereuse explication verbale? Telle est la question à laquelle répondent les diverses théories de la polarité, en proposant une explication d'ensemble des faits précédents.

Il est donc difficile de donner, dès maintenant, une définition précise de la polarité, sans préjuger de sa nature. Quelques conclusions semblent cependant se dégager du simple exposé des faits précédents.

(1°) La polarité, qui, par ses manifestations qualitatives, paraissait être uniquement une propriété de *direction*, identique pour tous les fragments d'un même individu, et comparable par conséquent à la polarité de l'aimant, se montre au contraire, dans ses manifestations quantitatives, variable suivant les relations du fragment avec l'*individu* primitif (niveau des sections, taille des fragments).

La polarité animale n'étant pas seulement une question de direction, on ne peut l'expliquer en faisant intervenir une orientation des particules protoplasmiques (théories particulaires).

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[1] Nous verrons ultérieurement d'autres manifestations de la polarité organique, relatives, non plus à des phénomènes de régénération, mais à des phénomènes physiologiques plus généraux. Ces faits seront exposés à propos de la théorie de Child, dont ils constituent les bases expérimentales les plus importantes.

La polarité se présente au contraire comme une propriété de l'individu dans son ensemble. Elle est plutôt une question de relations entre les parties de l'organisme, de corrélations : la polarité ne serait qu'un cas particulier, simple, de ces corrélations, le cas où elles s'exercent linéairement le long d'un axe morphologique[1]. Toute théorie de la polarité animale sera donc en même temps une théorie des corrélations entre les parties de l'organisme, une théorie de l'individualité.

(2°) L'examen des faits quantitatifs de polarité suggère l'idée d'une propriété, de "quelque chose," qui croîtrait de façon continue, d'une véritable *gradation*, le long de l'axe morphologique suivant lequel la polarité s'exerce. Des notions de cette nature sont en effet à la base de la théorie de Morgan, et surtout de celle de Child.

L'une des théories de la polarité, qui n'aurait peut-être plus qu'un intérêt historique si elle n'avait été reprise et défendue par J. Loeb, la théorie des substances formatives, repose cependant sur des notions d'un ordre tout différent, et il convient d'en rappeler ici les principes.

Iᵉ PARTIE: J. LOEB ET LA THÉORIE DES SUBSTANCES FORMATIVES.

La théorie des substances formatives, qu'on pourrait appeler aujourd'hui théorie hormonique, a été émise pour la première fois par Bonnet (1745), pour interpréter les faits de régénération chez *Lumbriculus*. Frappé par le fait que les tissus de cette Annélide sont capables, à tout niveau, de régénérer, soit une extrémité antérieure, soit une extrémité postérieure, Bonnet fait trois hypothèses: (1°) Il suppose que des germes de tête et des germes de queue existent dans toutes les parties du corps (on dirait aujourd'hui que les tissus sont totipotents). (2°) Que ces germes peuvent être éveillés (activés) par des substances spécifiques, formatrices de queue ou formatrices de tête. (3°) Que ces substances se meuvent dans des directions définies, les substances formatrices de tête d'arrière en avant, les substances formatrices de queue dans la direction opposée. Ces substances seraient normalement captées par les extrémités de l'animal. Dans les fragments, les substances formatrices de tête s'accumuleraient à l'extrémité antérieure, les substances formatrices de queue à l'extrémité postérieure, et y détermineraient la régénération.

Le botaniste allemand Sachs (1892) a énoncé cette hypothèse avec plus de force encore: pour lui, à tout organe morphologiquement défini du végétal correspond une substance spécifique déterminée se déplaçant dans une direction définie. C'est d'ailleurs aux végétaux que la théorie des substances organo-formatives a été le plus fréquemment appliquée: ainsi, elle a servi de guide à Goebel (1908) dans ses expériences de régénération chez les végétaux. On s'explique facilement que cette théorie, qui néglige les propriétés intrinsèques des différentes parties de l'individu, ait été plus aisément appliquée aux organismes végétaux, dont l'individualité semble très affaiblie, chez lesquels, notamment, la régénération ne

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[1] En ce sens, la polarité est un phénomène plus général que les phénomènes de régénération par lesquels elle se manifeste, et on conçoit qu'elle puisse exister là où la régénération fait défaut. On a même considéré la polarité comme une propriété générale de tous les organismes. Elle est en tous cas une propriété fondamentale de tous les œufs.

se présente pas avec ce caractère de restitution de la forme d'ensemble qu'elle affecte généralement chez les organismes animaux.

J. Loeb a donné à l'idée des substances formatives une vogue nouvelle en l'appliquant de nouveau aux animaux[1], et, cette fois, en se basant sur des faits d'observation. La théorie des substances formatives est restée constamment l'idée directrice de Loeb dans ses travaux sur la régénération, aussi bien animale que végétale.

A. EXPÉRIENCES DE LOEB SUR LA TUBULAIRE.

Les recherches sur la Tubulaire tiennent une grande place dans le développement de toutes les théories de la régénération[2]: les phénomènes sont là très nets et très rapides, l'expérimentation aisée. Mais l'interprétation des faits soulève plus de difficultés qu'ailleurs, car, d'une part, les phénomènes sont très sensibles aux variations des conditions externes, d'autre part, la polarité ne se présente pas, chez la Tubulaire, sous sa forme schématique. Il importe donc de donner d'abord un aperçu des faits de régénération chez la Tubulaire.

Les lois habituelles de la polarité exigeraient qu'un fragment de tronc de Tubulaire régénérât un polype à son extrémité orale, un stolon à son extrémité aborale. Or, si cela semble se produire dans les conditions naturelles, il n'en est pas de même dans les conditions expérimentales, celles du laboratoire, et l'on obtient alors très généralement (en proportions variables, d'ailleurs, suivant les espèces) des troncs portant un polype à chaque extrémité (hétéromorphose). Dans ce cas, cependant, les deux extrémités diffèrent en ce que le polype de l'extrémité orale se forme avant le polype aboral: c'est tout d'abord par cette différence entre les vitesses de développement des polypes oral et aboral que se manifeste la polarité dans cette forme où l'hétéromorphose est constante.

Des expériences classiques de J. Loeb (ligature du tronc; enfouissement d'une des extrémités dans le sable) ont montré que c'est bien le développement précoce d'un des polypes qui inhibe, pour ainsi dire, le développement du second. Le problème se décompose donc en deux parties: (1°) Pourquoi le développement du polype oral est-il plus précoce que celui du polype aboral? (2°) De quelle nature est l'action inhibitrice exercée par une des extrémités sur l'autre?

Quoi qu'il en soit, voici les faits observés au cours de la formation du polype, faits auxquels Loeb a attaché une grande importance, en les considérant comme une preuve positive de la théorie des substances formatives. Observés pour la première fois par Loeb, ces faits ont été minutieusement revus ensuite par Morgan (1901).

L'endoderme de la Tubulaire renferme des globules pigmentés en rouge, plus abondants dans les régions distales du tronc, ainsi que dans certaines parties des tissus endodermiques, qui constituent une sorte de cloison incomplète, séparant en deux parties la cavité gastro-vasculaire dans le sens longitudinal. Dans un

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[1] Les Hydraires sont, il est vrai, les animaux qui, par leur comportement général, rappellent le plus les végétaux.

[2] C'est à la Tubulaire qu'Allman a le premier appliqué le terme de "polarité," et c'est pour la Tubulaire que Loeb a créé le terme d'hétéromorphose.

fragment isolé de tronc, on voit apparaître très rapidement, dès la fermeture de la blessure (deux ou trois heures), une circulation de liquide, de plus en plus rapide, charriant des globules, d'abord clairs, puis colorés (cellules endodermiques ou fragments de cellules), de plus en plus nombreux. La cloison endodermique s'est rompue aux deux extrémités du fragment, et le courant parcourt toute la cavité, montant d'un côté, descendant de l'autre (Fig. 2).

Après un temps, variant de 12 à 24 heures, on voit apparaître, à l'extrémité distale du fragment, les ébauches des deux séries de tentacules du polype, sous la forme de deux rangées d'épaississements endodermiques pigmentés en rouge. Or, en même temps qu'apparaissent ces ébauches, les globules rouges disparaissent de la circulation.

D'où l'idée émise par Loeb que la régénération s'effectue aux dépens des matériaux mis en liberté dans la circulation, et que le pigment rouge, en particulier, est une véritable substance formative, nécessaire à la formation d'un polype. Loeb envisageait d'ailleurs, également, la possibilité d'un transport de matériaux de cellule à cellule, dans l'intérieur des tissus eux-mêmes. Driesch a repris intégralement les vues de Loeb et interprété les faits quantitatifs de polarité rappelés plus haut par la répartition inégale du pigment rouge dans les différentes régions du tronc.

Fig. 2.

Ces vues ont été vivement critiquées par T. H. Morgan (1901)[1], qui a fait valoir une série d'arguments très convaincants:

1°. La simultanéité entre le dépôt du pigment et le début de la régénération n'est pas toujours absolue: les épaississements endodermiques marquant l'ébauche des tentacules peuvent être apparents avant le dépôt du pigment rouge.

2°. Le pigment qui s'est accumulé dans la zone de régénération est rejeté, une fois la régénération achevée, par la bouche du polype (Morgan et Stevens, 1904).

3°. Certains troncs sont presque complètement dépourvus de pigment; or la régénération se produit chez ces individus avec la même vitesse, mais le polype formé est blanc.

4°. Si pendant le temps de circulation active, on recoupe l'extrémité aborale du fragment, pour provoquer la sortie des globules pigmentés, la régénération du polype oral n'est pas retardée de ce fait.

5°. La régénération du polype aboral se produit à un moment où il n'y a plus aucun pigment en circulation.

L'hypothèse du rôle formateur du pigment rouge semble donc bien devoir être abandonnée. On sait que l'on a attribué à des pigments un rôle analogue dans la question des localisations germinales; là aussi, il faut renoncer à un rôle formateur du pigment lui-même, qui ne fait que révéler des différences régionales dans l'œuf fécondé.

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[1] Morgan (1899) a formulé contre la théorie des substances formatives des critiques plus générales. Cette théorie lui paraît, notamment, difficilement applicable dans le cas, très fréquent chez les Invertébrés, où la régénération consiste, non pas en une croissance localisée, mais en un remaniement, en une refonte des anciens tissus, accompagnée de dédifférenciation, puis de re-différenciation (phénomène que Morgan a désigné sous le nom de "morphallaxis").

Nous n'avons aucune preuve de la migration de substances, spécifiques ou non, accompagnant le pigment, et s'opérant soit par la cavité endodermique, soit par les tissus eux-mêmes, durant les quelques heures qui précèdent la formation de l'ébauche du polype. Un tel transport vers l'extrémité distale du fragment se produit peut-être durant les stades de croissance du polype en régénération, mais il est alors la *conséquence*, non la *cause*, de la régénération en cette région.

D'ailleurs—et c'est l'objection la plus sérieuse qu'on puisse faire à la théorie des substances formatives se déplaçant dans des directions définies—l'existence d'une circulation[1], si elle est nécessaire au transport des substances, ne suffit pas à rendre compte de leur accumulation en un point déterminé: la circulation chez les animaux forme un cycle ininterrompu, et l'arrêt de certaines substances en un point défini ne peut provenir que d'une propriété locale préexistant à la section, de même qu'une hormone agit spécifiquement sur des tissus sensibles. La migration de substances dans une direction définie, et leur arrêt en une extrémité, qui constituent la base de la théorie de Bonnet, semblent impossible à concevoir autrement, avec les données actuelles de la physiologie animale. Peut-être n'en est-il pas de même, comme on le verra plus loin, chez les végétaux.

En résumé, la théorie des substances organo-formatives ne suffit pas à résoudre le premier problème posé par les faits de polarité chez la Tubulaire: pourquoi le développement d'un polype est-il plus précoce à l'extrémité orale qu'à l'extrémité aborale? Ce fait semble bien impliquer des différences entre les deux extrémités du fragment, réalisées antérieurement à la section.

La théorie est également impuissante à expliquer l'inhibition exercée par le polype oral sur le polype aboral: on ne peut invoquer une insuffisance de la quantité de substances formatives contenue dans le tronc pour la formation simultanée de deux polypes, car, si l'on place une ligature au milieu du fragment, les deux polypes se développent simultanément; de même, si l'on découpe le tronc considéré en de nombreux petits fragments, chacun de ceux-ci est capable de régénérer un polype.

B. Travaux de Loeb sur *Bryophyllum calycinum*.

Ces travaux ont été exposés par Loeb (1926) dans son dernier livre, auquel je renverrai pour le détail des faits. Les végétaux étant d'ailleurs en dehors du cadre de cet article, j'insisterai seulement sur quelques points, notamment ceux qui semblent révéler des différences fondamentales entre les phénomènes de régénération et de polarité chez les végétaux et chez les animaux.

L'idée directrice de Loeb est que la régénération est fonction de la quantité de substances nutritives mises à la disposition du régénérat: le poids sec de tissu (bourgeons, racines) régénéré est proportionnel, dans des conditions et un temps donnés, à la masse de tissus anciens, dont la synthèse chlorophyllienne fournit les matériaux nécessaires à la croissance du régénérat; c'est ce que Loeb appelle

[1] Le courant ciliaire observé par Loeb chez la Tubulaire semble être un fait banal chez les Coelentérés. Child a insisté sur la grande importance d'un tel courant, déterminant une certaine pression interne et la turgescence du corps de l'animal, dans la régénération et la croissance chez le Cérianthe.

la "relation de masses." On voit tout de suite que Loeb a seulement en vue les phénomènes de *croissance* qui accompagnent la régénération, et on pourrait appeler ce nouvel aspect de la théorie des substances formatives, *théorie nutritive* de la régénération.

Ces phénomènes de croissance constituent en effet l'essentiel dans la régénération végétale. Ce qu'on appelle régénération chez les végétaux n'est en somme que le développement de germes préformés (bourgeons), dont la croissance était jusque là arrêtée. On ne trouve, chez les végétaux, rien de semblable aux phénomènes, qui sont essentiels dans la régénération animale, comme la formation d'un blastème de régénération aux dépens de cellules indifférenciées ou dédifférenciées, ou les remaniements histologiques profonds de la régénération par "morphallaxis," tous faits qui prennent place immédiatement après le traumatisme, ont pour origine la section elle-même, et d'où dépend toute la régénération ultérieure[1].

La "régénération" chez les végétaux consiste uniquement, comme les travaux de Loeb le montrent bien, en une modification, consécutive au traumatisme, du système de répartition et de migration des substances nutritives, d'où dépend la croissance des différentes parties. Ainsi, si l'on isole de la tige une feuille de *Bryophyllum calycinum*, les substances nutritives qu'elle synthétise, qui, auparavant émigraient dans la tige, s'accumulent dans la feuille, et y déterminent le développement de bourgeons, primitivement dormants, situés dans chaque échancrure du bord de la feuille, et capables de régénérer une plante entière.

Loeb a consacré plusieurs chapitres de son livre à l'examen du "caractère polaire" de la régénération.

Pour ce qui est de la polarité qualitative, il renonce à expliquer le développement de bourgeons à l'extrémité distale et de racines à l'extrémité proximale d'un rameau par les différences de nature chimique existant entre la sève descendante et la sève montante, la première étant supposée transporter des substances formatrices de racines, la seconde des substances formatrices de bourgeons. Il obtient en effet, dans certaines conditions, au cours de ses expériences très variées et toutes ingénieuses, le développement de racines aux dépens de la sève montante, et, dans d'autres cas, le développement de bourgeons aux dépens de la sève descendante. Ces faits, et d'autres, comme le développement simultané de racines et de bourgeons aux dépens des mêmes sucs nutritifs, dans une feuille isolée, lui paraissent être de forts arguments "en faveur de la théorie qui attribue la polarité aux tissus et contre la théorie des hormones." Loeb conclut donc que si la sève ascendante forme généralement des bourgeons, c'est qu'elle rencontre d'abord les ébauches de bourgeons, tandis que la sève descendante rencontre d'abord des ébauches de racines.

Quant aux manifestations quantitatives de la polarité, elles semblent être, d'après les expériences de Loeb (chap. VII), beaucoup moins nettes que chez les

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[1] Il y a intérêt—nous reviendrons plus loin sur cette notion—à distinguer dans la régénération deux phases : une *phase préliminaire*, une *phase de croissance*, qui relèvent de mécanismes différents. Chez les végétaux, la première phase n'existe pas, ou, si l'on veut, se place dans le cours même de la croissance normale, dès avant le traumatisme qui déclanche la régénération.

animaux. Ainsi, dans la régénération à partir de nœuds isolés d'une même tige de *Bryophyllum*, le rapport de la masse de tissu régénéré à la masse de tissu ancien (qui mesure la rapidité de la régénération) est indépendant de la position du nœud sur la tige. Si au lieu de nœuds isolés, on coupe des fragments de tige contenant plusieurs nœuds, on retrouve encore le même rapport.

Mais, dans ce dernier cas, la polarité de la tige se manifeste par une inégale répartition de la sève nutritive entre les bourgeons des différents nœuds, les bourgeons apicaux croissant seuls, au détriment des bourgeons proximaux.

Pour Loeb, cette dernière manifestation de la polarité ne serait que secondaire : au début, tous les nœuds se développent également; puis, secondairement, les bourgeons apicaux et les racines proximales prennent le dessus, et la croissance s'arrête aux nœuds intermédiaires. Cet arrêt de croissance dans les régions moyennes du fragment de tige serait dû à une influence inhibitrice exercée par les parties à croissance rapide des deux extrémités. Les organes en voie de croissance rapide produiraient un appel de sève, une sorte d'effet de succion, dont le résultat serait de priver de nourriture les autres parties : tel serait le mécanisme de leur action inhibitrice[1].

Il reste à trouver la cause de la première différence de croissance entre les bourgeons proximaux et les bourgeons apicaux, différence qui ira ensuite en augmentant grâce à l'effet de succion exercé par les bourgeons apicaux. Cette différence initiale dans les vitesses de croissance pourrait être due à des différences intrinsèques de métabolisme entre les différents niveaux de la tige, comme celles qui ont été, ainsi qu'on le verra, mises en évidence par Child. Pour Loeb, le phénomène est dû uniquement au sens des courants de sève : du fait des sections, "il se réunit plus de sève aux deux extrémités de la tige que dans la région intermédiaire." "Il en résulte une légère accélération du développement des racines à la base et des bourgeons au sommet. Secondairement cette formation détermine un afflux vigoureux de la sève de toute la tige en ces deux régions, ce qui arrête le développement dans les nœuds intermédiaires."

Ainsi, la théorie des substances formatives, sous sa forme nouvelle, basée sur le sens des courants de sève et l'accumulation de substances nutritives qui en résulte, semble pouvoir rendre compte d'un certain nombre de faits dans la régénération végétale. Nous avons vu cependant son insuffisance à l'égard de la régénération animale.

Cette opposition est due sans doute à ce que la répartition des substances nutritives entre les divers organes se fait par des mécanismes bien différents chez les animaux et chez les végétaux. La circulation chez les végétaux est plutôt, autant qu'on le sache, une migration lente de substances nutritives, d'un ordre

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[1] Cette action inhibitrice exercée par les organes en voie de croissance, qui semble un fait assez général, est un des points les plus intéressants que les expériences de Loeb aient mis en évidence.

Dans ses mémoires préliminaires, Loeb avait fait intervenir des substances inhibitrices spécifiques—inconnues, naturellement—émises par le bourgeon terminal et transportées par la sève descendante dans les bourgeons inférieurs. Il paraît avoir complètement renoncé, dans son livre, à ce genre d'explication, pour proposer l'explication nutritive donnée plus haut.

de grandeur voisin de celui des phénomènes de croissance. Elle ne peut être comparée à la circulation, formant un cycle rapide et fermé, des animaux, chez lesquels les substances nutritives du milieu intérieur sont ainsi rapidement *mises simultanément à la disposition de toutes les parties de l'individu*. Aussi, conçoit-on que les explications fondées sur ces phénomènes de migration chez les végétaux soient difficilement applicables aux animaux.

CONCLUSIONS DE LA Iᵉ PARTIE.

1⁰. Loeb, dans ses derniers écrits, semble bien répudier la théorie des substances organo-formatives sous sa forme primitive: substances spécifiques, formatrices ou inhibitrices, circulant dans des directions déterminées. Il renonce à expliquer les manifestations qualitatives de la polarité chez le *Bryophyllum* par l'intervention de substances organo-formatives.

2⁰. Les expériences de Loeb sur *Bryophyllum calycinum* montrent que, chez les végétaux, le sens des migrations des substances nutritives, leur arrêt en certaines régions, peuvent déterminer des phénomènes de croissance dans certaines parties, les inhiber dans d'autres. Toutes les corrélations de croissance chez les végétaux relèvent peut-être de cette théorie nutritive. Il resterait d'ailleurs à trouver la cause de ces courants de sève.

3⁰. Il ne s'agit, dans la régénération végétale, que de la croissance de parties préformées. Dans la régénération animale, au contraire, les phénomènes de croissance se trouvent déterminés au cours d'une phase préliminaire de la régénération et par des mécanismes dont la théorie des substances formatives semble bien ne pouvoir rendre compte[1]: on a vu que l'application de cette théorie à la régénération des polypes de Tubulaire ne pouvait être acceptée.

Étant donnée l'allure toute différente de la circulation chez les animaux et chez les végétaux, il paraît difficile de faire intervenir, même dans la phase de croissance de la régénération animale, des mécanismes semblables à ceux qui sont invoqués par Loeb pour le *Bryophyllum*. Les phénomènes de transport qui se produisent dans l'organisme animal (par exemple, lors de la croissance d'un régénérat aux dépens de matériaux provenant des tissus anciens) diffèrent sensiblement de la migration des sucres dans un végétal. Ces phénomènes de transport ne peuvent d'ailleurs être considérés comme la cause, mais bien comme la conséquence de la croissance du régénérat.

IIᵉ PARTIE: LES THÉORIES DE CHILD.

INTRODUCTION: LA THÉORIE DE LA POLARITÉ DE T. H. MORGAN.

T. H. Morgan, à qui l'on doit la découverte de nombreux faits dans le domaine de la régénération, est certainement l'auteur qui a eu sur le sujet les idées les plus justes et les plus compréhensives. Nous avons déjà vu qu'il fut le premier à saisir

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[1] En particulier, l'explication donnée par Loeb de l'action inhibitrice exercée par un organe à croissance rapide ne peut s'appliquer à l'inhibition du polype aboral chez la Tubulaire, car, dans ce cas, ce n'est pas la croissance du polype aboral qui est arrêtée, mais les phénomènes préliminaires de sa régénération qui n'ont pas lieu.

l'intérêt de ce que nous avons appelé les "manifestations quantitatives" de la polarité, et qu'il a toujours combattu la théorie des substances formatives, qui néglige systématiquement l'interprétation de ces faits.

A la suite d'une étude expérimentale très serrée de la régénération chez les Tubulaires, Morgan (1901-1908) a été amené à formuler une théorie de la polarité, qui, quoique vague et surtout spéculative, constitue une introduction aux idées qui ont été ultérieurement développées par Child, et portées par lui sur le terrain expérimental.

La base de la polarité chez la Tubulaire est, pour Morgan, l'existence de *variations dans les qualités intrinsèques des tissus* aux différents niveaux du tronc. Ces différences se manifesteraient d'abord morphologiquement par des variations dans l'épaisseur des parois d'une extrémité à l'autre, et par les caractères histologiques des cellules, mais les différences essentielles seraient d'ordre chimique, chaque niveau étant caractérisé par sa composition chimique, qui varierait graduellement d'une extrémité à l'autre. Le tronc de la Tubulaire présenterait ainsi une véritable stratification de substances ("stratification," "gradation of materials").

Morgan n'apporte évidemment pas grande précision sur ces variations de composition chimique avec le niveau. Ces variations porteraient sur les proportions relatives de certaines substances, les unes étant plus abondantes au pôle oral, les autres au pôle aboral. Mais les substances en question ne peuvent être considérées comme des substances "organo-formatives" qui seraient mises en réserve, en quantité variable suivant le niveau, pour être utilisées lors de la régénération : la gradation de substances supposée par Morgan résulterait de la direction spéciale qu'a prise la différenciation histologique à chaque niveau ; elle ne serait que la marque de cette différenciation.

Cette *gradation de substances* dans le tronc de la Tubulaire serait l'origine des variations polaires dans la vitesse de régénération.

La vitesse de formation des polypes oraux, de même que celle des polypes aboraux (après ligature de l'extrémité orale du fragment) décroît régulièrement lorsqu'on déplace le niveau de la section de l'extrémité apicale à l'extrémité basale. Mais le niveau de la section n'est pas le seul facteur qui intervient pour déterminer la vitesse de régénération. En effet, si un fragment de tronc *AD* (Fig. 3) est ligaturé à ses deux extrémités, puis coupé en son milieu, la formation du polype oral en *C* précède un peu celle du polype aboral *B*, bien que ces deux polypes correspondent au même niveau du tronc primitif. Ce fait indiquerait, suivant Morgan (1905), une influence du sens de la gradation dans le tronc, le polype qui se développe dans le sens de cette gradation ayant l'avantage sur celui qui se développe en sens inverse.

Fig. 3.

Par quel mécanisme s'exerce cette influence du sens de la gradation ? et par quel mécanisme s'établit la gradation elle-même ? Ces mécanismes seraient ceux
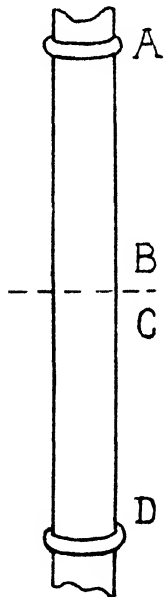
mêmes des corrélations, des relations entre les parties[1]; et ils mettraient en jeu, essentiellement, les propriétés d'irritabilité et de conductibilité du protoplasme[2].

A mon avis, un rapprochement s'impose entre ces vues qu'émettait Morgan dans ses derniers écrits sur la régénération (l'idée d'une gradation continue dans la différenciation protoplasmique aux différents niveaux, et cette conception des corrélations basée sur l'irritabilité fondamentale du protoplasme) et les notions qui sont à la base des idées de Child et que nous allons exposer maintenant.

LES THÉORIES DE CHILD.

C. M. Child, professeur à l'Université de Chicago, a été conduit, par de nombreux travaux expérimentaux sur la régénération chez les Invertébrés inférieurs, à élaborer, depuis 1910, un système théorique très cohérent, qui constitue certainement la tentative la plus poussée qui ait été faite pour donner une interprétation de l'ensemble des phénomènes de régénération. Par certains côtés, ces théories débordent même de beaucoup le domaine de la régénération, pour toucher à ceux de la reproduction asexuée, de la morphogénèse, de l'hérédité. Enfin, à l'heure actuelle encore, de nombreux mémoires, dus à Child ou à ses élèves, apportent chaque année de nouveaux faits qui confirment ou étendent ses théories.

Il est indispensable d'exposer tout d'abord l'ensemble des idées théoriques de Child sous une forme logique, avant d'entrer dans le détail des faits expérimentaux qui les justifient ou les vérifient, en quelque sorte *a posteriori*. Les bases théoriques du système de Child peuvent se résumer en un certain nombre de notions, comme le "gradient métabolique," l'"âge physiologique," la "dominance physiologique," qui, d'ailleurs, sont étroitement liées.

A. LA NOTION DE "GRADIENT PHYSIOLOGIQUE."

A la base du système de Child, comme à la base de toute théorie qui prétend donner une explication des phénomènes de régénération, se trouve une conception de l'individualité et des corrélations organiques.

Les corrélations, suivant Child, s'exercent en vertu d'une sorte de *hiérarchie* qui existe entre les différentes parties de l'organisme; certaines parties "dominant," tenant les autres sous leur dépendance. Le principe de cette hiérarchie est d'ailleurs purement physiologique: les parties dominantes sont celles qui ont la plus grande activité physiologique, activité qui se traduit, par exemple, par le taux du métabolisme respiratoire; elles dominent les autres parties en maintenant chez celles-ci une activité physiologique plus faible.

Dans le cas d'un organisme où les corrélations se manifestent par ce qu'on appelle une "polarité" suivant une certaine direction[3], les différents échelons de la hiérarchie physiologique sont disposés linéairement, suivant l'axe de la polarité

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[1] "Even the differentiation of the different regions must be supposed to be due to their relation to neighbouring regions" (Morgan, 1908).

[2] "Further analysis has led me to think that behind this relation (of the parts to each other) there is a more subtle one and that irritability is the physiological factor that regulates the behaviour of the cells in development and in regeneration" (Morgan, 1908).

[3] La théorie n'a guère été appliquée qu'aux êtres ou aux organes ainsi polarisés.

(qui est également un axe morphologique), les parties dominantes étant à une extrémité (la tête chez une Planaire, par exemple), les parties les plus dominées à l'autre extrémité. C'est l'ensemble de ces parties, graduellement hiérarchisées au point de vue physiologique, et disposées suivant un axe morphologique, que Child a appelé une échelle, un *"gradient" axial métabolique* ou *physiologique*.

Le fondement expérimental de cette notion est dans l'existence, dont nous verrons plus loin la démonstration, de *variations continues*, d'une extrémité à l'autre de l'axe morphologique, pour des *propriétés physiologiques* diverses[1]. Mais, dans la conception du gradient de Child, il se superpose à ces faits d'ordre physiologique, qui sont indiscutables, l'hypothèse de leur intervention dans le mécanisme des corrélations: l'idée de la "dominance" liée à une activité métabolique élevée.

Pour Child, d'ailleurs, toutes les différences, d'ordre qualitatif ou quantitatif, entre les parties d'un organisme polarisé, résulteraient des variations quantitatives d'une seule variable, le taux de métabolisme, ou l'activité physiologique. Les différences morphologiques ou histologiques elles-mêmes, dont Morgan fait la base de la polarité, ne seraient que des conséquences de différences quantitatives dans le taux du métabolisme.

B. La notion d'"âge physiologique."

Des différences dans le degré d'activité physiologique existent, non seulement entre les parties d'un même organisme, mais entre des organismes pris dans des conditions physiologiques différentes (âge, état de nutrition, état fonctionnel, etc.).

Or, les cellules jeunes sont caractérisées par une faible différenciation cytoplasmique, une activité respiratoire élevée, une grande perméabilité de leurs membranes, et, au point de vue morphogène par des potentialités très étendues: ce sont là les caractères des parties dominantes du gradient. Les parties séniles sont au contraire très différenciées histologiquement, ont un cytoplasme encombré d'enclaves, un taux de métabolisme faible, et des potentialités restreintes: telles sont les parties dominées d'un gradient physiologique. D'où l'introduction par Child de la notion d'âge physiologique, variant en sens inverse de l'activité physiologique.

Cet âge physiologique est, dans une certaine mesure, indépendant de l'âge réel, car la vie des organismes présenterait une série de cycles de "sénescence" suivis de "rajeunissement." Ainsi, la reproduction asexuée, la régénération d'un individu aux dépens d'un fragment, seraient accompagnées d'un rajeunissement physiologique. La fécondation serait un rajeunissement de l'organisme sénile qu'est l'ovule mûr. Pour une cellule glandulaire, la phase d'élaboration, de mise en charge, serait une phase de sénescence, la phase de sécrétion correspondant à un rajeunissement.

Je n'insisterai pas plus sur ces idées, exposées par Child dans son livre *Senescence and Rejuvenescence* (1915 *a*). Elles sont un peu en dehors de notre sujet, et, si elles permettent le rapprochement de nombreux faits biologiques et éthologiques, elles sont en partie purement spéculatives et n'ajoutent rien aux

[1] Les manifestations quantitatives de la polarité (voir l'Introduction) constituent déjà des variations de cette nature.

faits eux-mêmes: l'existence de variations de l'activité métabolique au cours des cycles fonctionnels ou reproducteurs.

C. NATURE DE LA "DOMINANCE PHYSIOLOGIQUE." ORIGINE DU GRADIENT.

L'influence exercée par les parties dominantes du gradient sur les parties subordonnées se traduit par le maintien dans ces dernières d'une activité physiologique inhibée, d'un métabolisme bas, et, corrélativement, d'un certain degré de différenciation histologique (bref, d'un certain état de "sénescence"). Par quel mécanisme s'exerce cette action des parties dominantes?

Pour Child, il y aurait à travers le protoplasme, *transmission*, suivant l'axe du gradient, d'une *excitation*, d'un "stimulus," d'une onde immatérielle, des régions dominantes vers les régions à faible métabolisme. Ce stimulus, d'origine externe, frapperait d'abord les parties dominantes—qui sont les plus "susceptibles," ainsi qu'on le verra plus loin (IIIe partie)—y produisant une excitation physiologique, une élévation du métabolisme. De là, cette excitation se propagerait vers les régions dominées, mais en présentant une décroissance continuelle de son intensité, un *décrément*, dû à une sorte d'inertie du protoplasme. Cet affaiblissement du stimulus expliquerait la diminution continue de l'activité physiologique qu'il détermine, diminution qui caractérise le gradient physiologique. A une certaine distance de la partie dominante, l'intensité de l'excitation deviendrait nulle: l'étendue du gradient est donc limitée suivant son axe, et les parties de l'organisme qui peuvent se trouver au delà de cette limite échappent à la dominance.

Le maintien du gradient métabolique résulterait donc du jeu des propriétés fondamentales d'*irritabilité* et de *conductibilité* de tout protoplasme vivant soumis à une excitation extérieure.

Les mêmes considérations permettent de concevoir l'origine d'un gradient comme résultant d'une *excitation extérieure localisée*, frappant un protoplasme primitivement isotrope. La répétition des mêmes phénomènes finirait par produire une différenciation permanente, physiologique et morphologique, suivant l'axe de transmission[1], différenciation qui serait la base de la polarité (cf. Child, 1915 *b*).

Ces vues paraîtront peut-être bien spéculatives. Pourtant, nous savons que la polarité primitive de l'œuf peut être déterminée par la pesanteur, que sa symétrie est déterminée par le point d'entrée du spermatozoïde. Child (1920 *a*) rappelle que dans le développement de l'œuf de certaines Fucacées, d'après Winkler (1900) et Kniep (1907), et dans le développement de la spore d'*Equisetum*, d'après Stahl (1885), la polarité est déterminée également par les facteurs externes (ici, la direction de la lumière). Enfin, Child (1925) a récemment montré que la polarité de l'œuf des Hydraires *Phialidium gregarium* et *Stomotoca atra* est déterminée par ses relations trophiques avec l'organisme maternel: l'extrémité dominée du gradient de l'œuf correspond au pôle par lequel l'oocyte est attaché dans la gonade, l'extrémité dominante au pôle libre. Ces faits, et d'autres analogues, nous montrent

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[1] Child compare cette production de structures permanentes, irréversibles, par une excitation répétée, à l'hypertrophie fonctionnelle du tissu musculaire, et aux phénomènes de mémoire dans le fonctionnement du système nerveux.

que la polarité d'un organisme peut être déterminée par un facteur externe, agissant de façon asymétrique.

Quant au mode particulier d'excitation-transmission qui serait à l'origine du gradient métabolique, on peut le comparer à la transmission de l'influx nerveux, ou, dans le cas des formes à système nerveux peu différencié, au mécanisme des corrélations par voie "aneurale" mis en évidence par Wintrebert (1921) chez les embryons de Vertébrés, et connu également dans la coordination des mouvements ciliaires chez les Infusoires et les Cténophores.

Child (1915 *b*) considère en effet l'apparition du système nerveux comme la manifestation morphologique la plus parfaite de l'établissement d'un gradient physiologique[1]. Il y a longtemps, d'autre part, que Ch. Richet a considéré l'intensité du métabolisme d'un tissu comme étant principalement sous la dépendance du stimulus nerveux reçu; et la théorie de Child n'est, en ce sens, qu'une application de la théorie de Richet aux organismes polarisés.

Le mode de coordination des mouvements ciliaires offre des points de ressemblance remarquables avec le mécanisme des corrélations invoqué par Child: le mouvement débute par un pôle et se propage dans un sens déterminé; chaque cil a son mouvement commandé par le cil précédent et commande le mouvement des suivants. Cette hiérarchie graduelle pour la commande du mouvement est très comparable à la dominance physiologique pour la commande du métabolisme.

En dernière analyse, le gradient physiologique aurait donc pour base une diminution d'irritabilité depuis les régions dominantes jusqu'aux régions inférieures, et une conductibilité élective des excitations dans cette même direction. En tous cas, les corrélations, telles que les conçoit Child, dans un organisme polarisé, seraient plus comparables aux corrélations nerveuses ou aux corrélations aneurales qu'aux corrélations par voie humorale ou hormonique.

D. Application à la reproduction asexuée. L'"isolement physiologique."

Lorsqu'une partie primitivement dominée du gradient physiologique se trouve, pour une raison quelconque, soustraite à l'influence des parties dominantes, la cessation des corrélations ("isolement physiologique") se traduit, dans cette région, par une augmentation du taux de métabolisme, une "stimulation," un "rajeunissement," et la région ainsi stimulée peut devenir l'origine d'un nouveau gradient.

Dans la reproduction asexuée, l'*isolement physiologique* peut résulter, soit d'une *atténuation* de la dominance dans l'individu primitif, par diminution de l'activité physiologique dans la partie dominante de son gradient; soit de ce que, par suite de la croissance et de l'allongement du corps suivant l'axe du gradient, les parties les plus éloignées de la région dominante se trouvent portées au delà de sa limite de dominance.

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[1] La polarité d'une Planaire serait en somme de même nature que la polarité du neurone. La polarité du neurone se manifeste tout d'abord, fonctionnellement, par une conductibilité élective dans le sens cellulifuge, mais elle se manifeste également par des phénomènes de régénération, et on a pu mettre en évidence dans les nerfs (Tashiro, 1913 *b*; Child, 1914 *e*) l'existence d'un véritable gradient métabolique.

Ainsi, chez les Planaires scissipares, comme *Planaria dorotocephala* Woodworth, il s'individualise à l'extrémité postérieure du corps, par isolement physiologique, des régions à métabolisme élevé, qui deviennent les têtes de nouveaux gradients, et qui constituent, dès avant la séparation effective, et même avant toute différenciation morphologique, des individus physiologiquement indépendants, de nouveaux zooïdes. *Planaria dorotocephala* est ainsi normalement composée de deux, trois, quatre ou cinq zooïdes (Fig. 4). La scissiparité résulte d'une déchirure des tissus due aux réactions motrices indépendantes des deux premiers zooïdes[1].
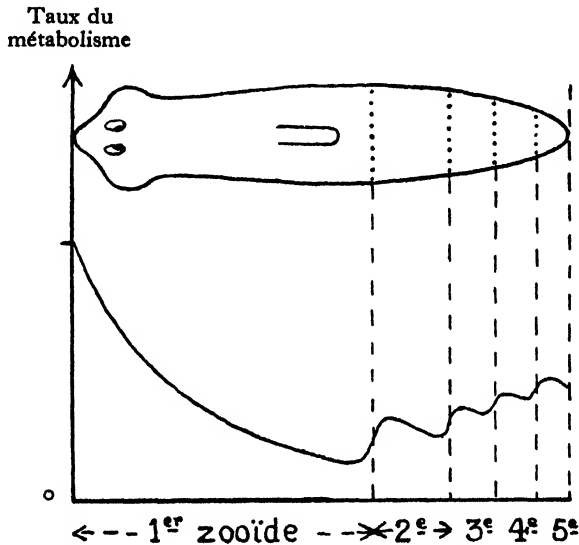
Fig. 4.

D'après cette théorie, la reproduction asexuée serait sous la dépendance de la taille atteinte par l'organisme qui se divise[2], et, d'autre part, de l'état physiologique des zooïdes; toute cause tendant à atténuer l'activité physiologique du premier zooïde, ou à exalter celle du second, favorisant la scission.

Ainsi, Child (1910) a montré—et Vandel (1921) a vérifié le fait—l'influence favorisante exercée sur la scission par la décapitation du premier zooïde: cet effet est obtenu quelques jours après la section, alors que la nouvelle tête régénérée possède une activité physiologique encore faible. Beaucoup de facteurs externes influencent la scissiparité en exerçant une action différentielle sur le métabolisme des deux zooïdes: le second zooïde, étant physiologiquement plus jeune que le premier, s'acclimate plus rapidement aux influences extérieures défavorables (cf.

[1] Vandel (1921), dans son étude de la scissiparité chez les Planaires européennes (*Polycelis cornuta* Johnson, *Planaria alpina* Dana, etc.), a confirmé ce dernier fait. Il met en doute, cependant, l'existence de zooïdes à limites bien tranchées, pour cette raison que la déchirure se produit suivant une ligne quelconque, non déterminée à l'avance. On peut faire remarquer, pourtant, que la limite des deux zooïdes pourrait être bien déterminée, sans être par là même une ligne de moindre résistance mécanique.

[2] Le bourgeonnement, chez les Oligochètes Naïdimorphes, ne se produit, d'après L. Dehorne (1916), que lorsque l'individu-souche a dépassé un certain nombre de segments, qui est fixe et caractéristique de l'espèce.

IIIe partie). Ainsi s'interprète l'action favorisante exercée sur la scission par le séjour prolongé dans les anesthésiques dilués (alcool à 1,5 %), par les températures extrêmes, ou par l'inanition[1].

Enfin, l'existence de plusieurs zooïdes physiologiques chez *Planaria dorotocephala* peut être démontrée par les différentes méthodes qui mettent en évidence les gradients physiologiques, et qui seront exposées dans la IIIe partie. Elle se manifeste aussi par des différences quantitatives polaires dans les phénomènes de régénération: la vitesse de régénération de la tête dans des fragments de même taille provenant des différents niveaux du corps décroît régulièrement à partir de la tête jusqu'à l'extrémité postérieure du premier zooïde; mais, aux niveaux plus postérieurs, comprenant les zooïdes physiologiquement plus jeunes, la vitesse de formation de la tête atteint et dépasse même celle du niveau de la tête du 1er zooïde (Child, 1906 *a*).

E. APPLICATION À LA RÉGÉNÉRATION.

1°. *Autodifférenciation des parties distales.*

Le développement d'un individu à partir d'un fragment doit se faire, comme le développement normal ou la reproduction asexuée, sous la direction d'une partie à métabolisme élevé, qui sera l'extrémité dominante du gradient dans le nouvel individu.

En fait, dans la régénération d'un organe, c'est l'extrémité distale de l'organe qui est d'abord mise en place, les parties intermédiaires n'apparaissant qu'ensuite. Ainsi, la régénération antérieure le long d'une section transversale de Planaire différencie tout d'abord une tête, quel que soit le niveau de la section, quels que soient par conséquent les organes manquants. Ces organes se différencient ensuite progressivement, à partir de la nouvelle tête formée.

Déjà T. H. Morgan (1904) avait insisté sur cette loi. Pour Child, la différenciation des organes intermédiaires dans le sens apico-basal résulte de l'établissement du gradient dont la nouvelle tête est la partie dominante. Quant à la tête, étant physiologiquement indépendante des autres niveaux, elle s'autodifférencie, en quelque sorte, dans les premiers tissus régénérés.

2°. *Établissement d'un nouveau gradient.*

Le traumatisme provoque dans les cellules de la surface de section une stimulation, une augmentation de métabolisme (qui correspond sans doute, morphologiquement, à des phénomènes de dédifférenciation). Ce stimulus va être transmis, avec un certain décrément, dans tout le fragment en régénération, et la surface de section va tendre à devenir la région dominante d'un nouveau gradient.

Mais l'ensemble du fragment possède déjà un certain taux de métabolisme, résultant de la place qu'il occupait dans l'individu primitif. Si ce métabolisme de l'ensemble du fragment se trouvait être plus élevé que celui de la surface de

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[1] Vandel (1921) insiste sur le fait que, si ces différents moyens favorisent effectivement la scission dans une espèce de Planaire qui la présente normalement, ils sont cependant impuissants à la provoquer dans une espèce ou une lignée qui ne possède pas cette faculté, en quelque sorte, dans son génotype.

section stimulée, le nouveau gradient ne pourrait s'établir. Si la différence entre les deux taux de métabolisme est faible, le gradient qui s'établira sera peu marqué, inhibé en quelque sorte par le métabolisme élevé du fragment, et cette influence retentira sur la régénération. La nature du régénérat dépendra donc des valeurs relatives des taux de métabolisme du fragment et de la tranche de section.

Prenons avec Child l'exemple d'un fragment de *Planaria dorotocephala* limité par deux sections transversales, et appelons (x) la tranche antérieure de section, (y) la masse principale du morceau (Fig. 5). La "fréquence de la tête" (c'est-à-dire la proportion d'individus ayant régénéré une tête normale), qui mesure, comme on l'a vu dans l'Introduction, la qualité de la régénération dans un lot de Planaires, croîtra sous l'influence de toute cause tendant à élever le métabolisme de (x), et décroîtra sous l'influence d'une cause élevant le métabolisme de (y). Cette loi, que l'expérience vérifie, comme on le verra, est représentée par Child par la formule schématique:

$$\text{Fréquence de la tête} = \frac{\text{Taux de } x}{\text{Taux de } y}.$$

3°. *Régénération postérieure. Hétéromorphose.*

Dans un fragment limité par deux sections transversales, la tranche postérieure (z) (Fig. 5) est également l'origine d'un stimulus qui tend à établir un second gradient, opposé au gradient (x). Le développement de ce second gradient est généralement inhibé par le premier, car, d'une part, la région (x) a un métabolisme plus élevé que la région (z) (par suite de la position de ces sections dans le gradient primitif), et, d'autre part, le gradient (x) a l'avantage de se développer dans le sens même de ce gradient primitif. Aussi la tranche (z) entre-t-elle sous la dominance de (x); et sa croissance ultérieure aboutit à la différenciation d'une queue[1].

Pourtant, il arrive que, dans le cas de fragments très courts, la différence de métabolisme entre (x) et (z) étant très faible, les deux gradients puissent subsister indépendamment et que chacune des extrémités (x) et (z) régénère une tête: telle est l'interprétation que donne Child des faits d'hétéromorphose[2].

4°. *Stimulation des fragments.*

La section, qui produit l'isolement physiologique du fragment mis à régénérer, provoque dans l'ensemble de ce fragment une stimulation, une augmentation temporaire de métabolisme. Cette stimulation, qui a pu être décelée expérimentalement (Child, 1914 *a*; Hyman, 1916, 1923) (cf. IIIe partie), se produit pendant les premières heures qui suivent la section, le métabolisme passant par un maximum et revenant à son taux primitif environ 24 h. après la section.

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[1] On remarquera que la théorie de Child ne fait pas intervenir, comme la théorie des substances organo-formatives, un mécanisme double, un pour la formation des structures antérieures, un pour les structures postérieures. Suivant Child, toute partie physiologiquement isolée tend à produire une tête, et ne donne une queue que sous une influence inhibitrice.

[2] On savait depuis longtemps que les Planaires à deux têtes ne pouvaient être obtenues qu'à partir de fragments très courts. Morgan avait même indiqué que l'explication de ce fait était que d'une différence de niveau moins grande résultait une polarité moins marquée.

Cette disparition de la polarité dans les morceaux au-dessous d'une certaine taille montre bien que la polarité n'est pas une propriété intrinsèque de chaque particule protoplasmique, mais une question de relation entre les parties.

La valeur de cette augmentation de métabolisme dépend: (1°) de la taille des fragments: les gros fragments (1/2, 1/3 du corps) ne sont pas stimulés; les petits fragments le sont d'autant plus qu'ils sont plus petits; (2°) de la position du fragment dans l'individu primitif, les fragments correspondant aux parties inférieures du gradient étant plus stimulés que les fragments provenant des parties dominantes: la stimulation est d'autant plus forte que le métabolisme était auparavant plus inhibé.

Ainsi, la section du fragment provoque une "stimulation," non seulement dans les tissus de la tranche (*x*) (Fig. 5), mais aussi dans la partie principale (*y*) du fragment. Or, nous allons voir que, chez *Planaria dorotocephala*, c'est précisément pendant la période de stimulation de (*y*), c'est-à-dire pendant les premières 24 heures qui suivent la section, que s'établit, à partir de la section (*x*), le gradient du nouvel individu.
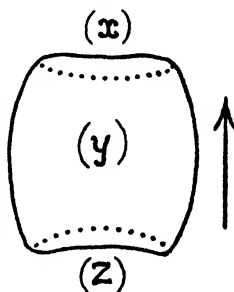
Fig. 5.

5°. Temps de "détermination de la tête."

De petits fragments *ABCD* (Fig. 6), limités par deux sections transversales, et prélevés dans la région postérieure du premier zooïde de *Planaria dorotocephala*, sont incapables de régénérer une tête normale (fréquence de la tête très faible), ce que peuvent faire les fragments plus gros *ABE*. Or, si, au lieu d'opérer simultanément les deux sections *AB* et *CD*, on fait la section *CD* un certain temps (variant de 12 à 18 heures) après la section *AB*, les fragments *ABCD*, par le fait même qu'ils sont restés, pendant cette courte période, en relation avec le morceau postérieur *CDE*, régénèrent alors un pourcentage très élevé de têtes normales (fréquence de la tête élevée) (Child, 1914 *b*). Il faut donc admettre que la nature du régénérat est déjà, en quelque sorte, déterminée en puissance, 18 h. après la section.
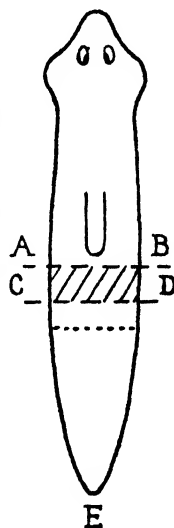
Fig. 6.

L'interprétation des résultats est la suivante: les petits fragments *ABCD* subissent une plus forte stimulation que les fragments plus gros *ABE*, et le métabolisme élevé de la région (*y*) inhibe dans ces petits morceaux l'établissement du nouveau gradient; il en résulte une fréquence de la tête très faible.

Cette expérience remarquable—qui a pu être répétée par L. H. Hyman (1916) dans des conditions analogues, sur l'Annélide *Lumbriculus*—nous montre que le nouveau gradient s'établit, chez *Planaria dorotocephala*, en moins de 18 heures, c'est-à-dire pendant la période de forte stimulation de la région (*y*). Le taux de métabolisme de (*y*) qui intervient dans le conflit entre (*x*) et (*y*), schématisé par la formule

$$\text{Fréquence de la tête} = \frac{\text{Taux de } x}{\text{Taux de } y},$$

n'est donc pas celui que possédait le fragment dans l'individu primitif, mais celui qui correspond à la période de stimulation.

6°. *Interprétation des expériences de régénération.*

Il résulte du schéma théorique précédent que la fréquence de la tête doit être en raison inverse du degré de stimulation de l'ensemble (y) du fragment: elle doit donc être d'autant plus faible que les fragments sont plus petits et que le niveau de la section est plus postérieur (dans l'étendue du ᵉʳ zooïde). C'est en effet le résultat donné par l'expérience (Child, 1911 *b*).

Si l'on compare entre eux des individus différents, la fréquence de la tête doit varier en raison inverse du métabolisme, c'est-à-dire en raison directe de l'âge physiologique. Elle augmente en effet avec l'âge des individus, et diminue au cours de l'inanition (Child, 1920 *b*).

Enfin, la conception théorique, schématisée par la formule

$$\text{Fréquence de la tête} = \frac{\text{Taux de } x}{\text{Taux de } y},$$

permet également d'interpréter les expériences relatives à l'influence, sur la fréquence de la tête, des agents externes: température (E. H. Behre, 1918), activité motrice (Child, 1920 *b*), anesthésiques et toxiques.

Prenons l'exemple du cyanure KCN. L'action de KCN dilué, pendant un temps très court, au moment de la détermination de la tête, produit une diminution de la fréquence de la tête dans des fragments constitués par le tiers antérieur du premier zooïde décapité, mais produit au contraire une augmentation de la fréquence de la tête dans les fragments constitués par le tiers postérieur du premier zooïde (Child, 1916 *a*; Buchanan, 1922). Ces résultats s'expliqueraient par l'action différentielle du réactif sur les parties (x) et (y): l'action inhibitrice de KCN porte surtout sur la région qui a le métabolisme le plus élevé; c'est-à-dire, dans les fragments antérieurs peu stimulés, sur la région (x) et, au contraire, dans les fragments postérieurs fortement stimulés, sur la région (y).

CONCLUSIONS DE LA IIᵉ PARTIE.

Pour Child, toutes les manifestations de la "polarité" dépendent donc de l'existence d'un "gradient physiologique," caractérisé par la gradation de l'activité métabolique d'une extrémité à l'autre de l'axe morphologique de l'organisme polarisé. Ce gradient tirerait son origine d'une influence extérieure elle-même polarisée et serait maintenu ensuite grâce à l'irritabilité plus grande d'une des extrémités (région dominante) et à la conductibilité du protoplasme suivant l'axe du gradient.

Cette définition physiologique de la polarité peut s'appliquer à tous les cas, notamment aux organismes dans lesquels la polarité ne se manifeste pas par des phénomènes de régénération: l'existence d'un gradient métabolique constitue donc la meilleure *définition de la polarité*.

La régénération dépend de l'établissement, dans le fragment, de la dominance des tissus de la section. Cette dominance est établie au bout de 24 heures, et ce phénomène constitue en quelque sorte une phase préliminaire de la régénération, dont la durée remarquablement courte concorde avec le caractère pseudo-nerveux des mécanismes qui seraient mis en jeu. Le métabolisme propre des tissus du

fragment—qui, pendant cette phase, subit du fait de la section une stimulation plus ou moins grande—tend à inhiber l'établissement du nouveau gradient. Ainsi, suivant la conception de Child, la régénération, loin d'être une restitution de la forme primitive *dirigée par les tissus anciens*, est un phénomène de croissance autonome, ayant pour point de départ la section et se produisant au contraire *malgré* les parties anciennes du fragment.

De même que, dans la régénération, la "détermination" de la tête précède son apparition, dans la reproduction asexuée, la différenciation physiologique des zooïdes précède leur différenciation morphologique.

En général, l'établissement d'un gradient physiologique précède les phénomènes morphologiques qu'il conditionne. Tels seraient le rôle et la signification des gradients physiologiques: ils détermineraient, dans la morphogénèse, le type d'organisation, de symétrie, la présence ou l'absence de certaines parties, tous les traits généraux de l'organisation, qui seraient indépendants des particularités spécifiques dans la constitution protoplasmique de l'espèce (Child, 1920 *a*).

IIIᵉ PARTIE: BASES EXPÉRIMENTALES DES THÉORIES DE CHILD.

Nous avons exposé dans la partie précédente l'ensemble des notions théoriques qui résument l'œuvre de Child et de ses collaborateurs. On a vu qu'elles découlaient toutes logiquement d'une conception originale des corrélations. Mais ce mode de présentation, inévitable étant donnée la nature du sujet, s'il a eu l'avantage de montrer la grande cohérence de ces conceptions, a pu laisser croire au lecteur que le système de Child était une construction arbitraire et entièrement *a priori*.

En réalité, toutes les hypothèses qu'implique la théorie de Child n'ont été faites que devant la nécessité d'interpréter de nouveaux faits, et leur vérification a constamment suggéré de nouvelles expériences. Aussi devons-nous maintenant donner au moins une idée de la somme considérable de travaux expérimentaux que représente l'œuvre de Child.

A. Bases expérimentales de la notion de gradient.

On a vu qu'un gradient physiologique était caractérisé par les variations graduelles de diverses propriétés physiologiques, le long d'un axe morphologique.

La propriété physiologique fondamentale, celle dont, suivant Child, dériveraient toutes les autres, est l'*activité métabolique*, mesurée par l'*intensité des échanges gazeux respiratoires*. Malheureusement, la mesure directe des échanges gazeux présente souvent de grandes difficultés techniques, et Child a eu plus fréquemment recours, pour la mise en évidence des gradients physiologiques, à des méthodes indirectes, qui reposent sur l'*action différentielle* d'un même agent physiologique à l'égard des différents échelons du gradient. Ces divers procédés indirects ne fournissent pas une mesure du métabolisme, mais ils permettraient de déceler des variations du taux de ce métabolisme, et les indications qu'ils donnent ont pu être contrôlées, dans des cas qui deviennent d'année en année plus nombreux, par la mesure directe des échanges gazeux.

1°. *Mesures directes du taux de métabolisme.*

La mesure des quantités de gaz carbonique dégagé ou d'oxygène absorbé par des fragments d'Invertébrés aquatiques offre de grandes difficultés : difficultés techniques tenant à la faible masse du matériel, à la vie aquatique des animaux ; difficultés de pesée. En outre, on a vu que des fragments isolés d'un organisme subissent, du fait même de la section, une augmentation du taux de leur métabolisme, et que le métabolisme mesuré quelques heures après la section est tout différent de celui du fragment dans l'individu.

D'autre part, des mesures directes ne peuvent donner que le métabolisme global du fragment, qui est la somme des métabolismes des différents tissus. Or, le taux du métabolisme varie avec la nature du tissu, et les divers tissus peuvent exister en proportions variables suivant le niveau. Ainsi, chez les Planaires, si l'activité métabolique décroît de façon continue, de la tête vers l'extrémité postérieure, dans les tissus ectodermiques, il n'en serait pas de même pour le tube digestif, qui présenterait son maximum de métabolisme dans la région du pharynx, c'est-à-dire vers le milieu du corps.

Malgré toutes ces difficultés, de nombreux essais ont été tentés par l'école de Child, surtout dans ces dernières années, pour fournir une preuve directe de l'existence de variations de métabolisme en rapport avec les gradients physiologiques.

(*a*) *Dégagement de gaz carbonique.* Le dégagement de CO_2 a été mesuré, soit par le temps nécessaire pour atteindre un certain pH dans le milieu, le pH étant évalué par la méthode colorimétrique ; soit par la méthode de Tashiro (1913 *a*), qui permet d'apprécier de très faibles quantités de CO_2, par la pellicule de carbonate de baryum qui se forme à la surface d'une goutte d'eau de baryte.

Robbins et Child (1920), utilisant la méthode colorimétrique, ont pu retrouver chez *Planaria dorotocephala* le phénomène de la stimulation et ses variations avec le niveau des fragments. Ils mettent en évidence une augmentation dans la production de CO_2, aussi bien dans les tissus régénérés que dans les tissus anciens, après 15 jours de régénération. Leurs résultats confirment et complètent ceux de nombreux mémoires antérieurs.

(*b*) *Consommation d'oxygène.* L. H. Hyman (1923), utilisant une modification de la méthode de Winckler pour le dosage de l'oxygène dissous dans l'eau, a pu montrer que l'absorption d'oxygène par des fragments de *Planaria dorotocephala* suivait bien toutes les lois de la stimulation exposées dans la partie précédente.

Des différences dans la consommation d'oxygène en rapport avec l'existence de gradients physiologiques ont été démontrées chez les Annélides (*Lumbriculus, Nereis*) par L. H. Hyman et Galigher (1921), dans l'Éponge *Granthia* par L. H. Hyman (1925), chez les Hydraires (*Tubularia*) par L. H. Hyman (1926).

2°. *Méthode de "susceptibilité différentielle."*

Child, dès ses premiers travaux sur la régénération, a été frappé de la relation qui existe entre les phénomènes de régulation et l'activité fonctionnelle des organismes (cf. Child, 1906 *b*). Cette idée l'a conduit à étudier l'influence des anesthésiques et des toxiques sur la régénération ; les actions différentielles observées au cours

de cette étude ont été l'origine de la conception du gradient physiologique. La méthode de susceptibilité aux réactifs, la première en date, est toujours restée la méthode fondamentale dans les travaux de l'école de Child.

La méthode de susceptibilité repose sur ce fait qu'en règle générale, les régions (ou les individus) à métabolisme élevé sont les plus sensibles à l'action des agents employés à dose mortelle, et qu'inversement, elles sont aussi les plus rapidement "acclimatées" à une concentration compatible avec la vie. Les agents toxiques peuvent donc produire, suivant leur concentration, des effets exactement opposés, mais leur action, à chaque concentration, est toujours différentielle pour les régions d'âges physiologiques différents.

La méthode se décompose donc en deux: (*a*) la méthode directe ou de *suscepti-bilité*, consistant en l'emploi de réactifs à dose mortelle; (*b*) la méthode d'*acclimatation*, impliquant l'emploi de doses plus faibles, non mortelles.

Le réactif qui a été le plus fréquemment employé par l'école de Child est le cyanure de potassium KCN. On connaît depuis longtemps l'influence de ce sel comme inhibiteur des oxydations. Loeb l'a fréquemment utilisé à ce titre, et il était naturel de s'adresser à son action pour déceler des différences dans l'intensité des oxydations. Mais des discussions se sont élevées récemment au sujet de cette action spécifique des cyanures (cf. Lund, 1918; Child et L. H. Hyman, 1919; Hyman, 1919 *a* et *b*), et Child et Hyman (1919) admettent que le rôle de KCN dans les expériences de susceptibilité est indépendant de son action spécifique comme inhibiteur des oxydations: Child et ses élèves ont obtenu en effet des résultats identiques à ceux que donne le cyanure en utilisant les réactifs les plus variés: des anesthésiques, comme l'alcool, l'éther, le chloroforme, le chlorétone, les alcaloïdes (caféine; M. A. Hinrichs, 1924 *a*), les uréthanes; des toxiques, le sublimé, le formol, des acides, des bases, des sels, des colorants vitaux. Ils ont également utilisé des agents physiques: l'action des températures extrêmes; l'action des rayons ultra-violets, ou celle des radiations visibles du spectre sur des animaux préalablement sensibilisés par l'éosine ou des colorants vitaux (M. A. Hinrichs, 1924 *b*; Child et E. Deviney, 1925). Enfin, la simple privation d'oxygène donne lieu à des phénomènes différentiels de même nature[1].

(*a*) Tous les réactifs précédents, employés à une concentration suffisante, produisent, en un temps, variable avec leur concentration[2], la mort des cellules et la désintégration des tissus. La désintégration des différentes régions du corps se produit, quel que soit l'agent employé, dans un ordre défini et toujours le même. Chez *Planaria dorotocephala*, par exemple, elle débute par la tête des zooïdes, puis gagne les régions marginales du corps en progressant dans le sens antéro-postérieur, révélant ainsi le gradient de susceptibilité de chaque zooïde.

[1] Dès 1912, A. Drzewina et G. Bohn avaient mis en évidence des différences de résistance à la privation d'oxygène aux différents niveaux, pour quelques organismes, Turbellariés (Drzewina et Bohn, 1912, 1913), Hydre (1916), et considéré ces phénomènes comme des manifestations de la polarité chimique.

G. Bohn a d'ailleurs exposé une théorie intéressante et originale de la polarité dans son livre *La forme et le mouvement* (1920), auquel je renverrai.

[2] Les concentrations utilisées varient pour le cyanure KCN par exemple, de la concentration moléculaire à la concentration 0,001 M, suivant le matériel.

La vitesse de désintégration dépendrait, pour une même concentration de réactif, de l'"âge physiologique" des individus. Child distingue, dans la pratique, cinq stades dans la désintégration d'une Planaire, et le dénombrement, à intervalles réguliers, des individus aux différents stades, dans deux lots de Planaires à comparer, permet de mesurer leur susceptibilité.

(*b*) La méthode d'acclimatation consiste à comparer, à intervalles réguliers, la proportion d'animaux survivants dans des lots d'individus ou de fragments à comparer, placés dans des solutions très diluées de toxiques ou d'anesthésiques.

Bien qu'*a priori* il semble que les nombreux agents utilisés dans les deux méthodes précédentes doivent agir par des mécanismes différents, il est frappant de constater la concordance remarquable des résultats qu'ils fournissent. Cette *non-spécificité* des agents employés, sur laquelle Child et ses élèves ont souvent insisté, suggère l'idée que l'action de ces agents dépend d'un facteur inhérent à l'organisme.

E. J. Lund (1917, 1918 *a*, 1918 *b*) et B. L. Lund (1918) ont critiqué la valeur de la méthode de susceptibilité en prétendant qu'elle ne pouvait indiquer que des différences de perméabilité, et que la perméabilité était une propriété des cellules, indépendante des oxydations. L'action des agents physiques (temperature, radiations) et de la privation d'oxygène, utilisée par Child, semble cependant exclure l'intervention de la perméabilité cellulaire. D'ailleurs, Child et L. H. Hyman (1919) critiquent, non sans quelque raison, la notion d'une perméabilité purement physique, qui serait une simple propriété de surface, complètement indépendante de l'activité métabolique du protoplasme.

3°. *Autres méthodes indirectes*.

(*a*) *Variations du pouvoir réducteur vis-à-vis de certaines substances* (Child, 1919, 1921, etc.). Les solutions de permanganate de potasse provoquent très rapidement la mort de petits organismes aquatiques. En même temps, le permanganate est réduit à l'intérieur des cellules, où il se forme un précipité brun d'oxyde MnO_2. Dans des solutions très diluées (N: 10000), le phénomène est plus lent et plus régulier: on observe alors, suivant les parties d'un même organisme, des différences dans la vitesse de formation du précipité et surtout dans la quantité totale de précipité formé. Le matériel peut ensuite être monté, et les différences d'intensité de la coloration brune obtenue apparaissent clairement.

Ces différences de coloration correspondent—pour les matériaux les plus variés qui ont été utilisés—au gradient physiologique, tel qu'il est mis en évidence par toutes les autres méthodes: les parties dominantes du gradient sont les plus intensément colorées. Si l'organisme est tué avant d'être plongé dans le perman-ganate, la coloration obtenue est uniforme.

(*b*) *Différences d'intensité dans la réaction du bleu d'indophénol*. Cette réaction, caractéristique de certaines diastases oxydantes, consiste en l'apparition d'un précipité de bleu d'indophénol à l'intérieur des cellules. La coloration bleue produite est plus intense au pôle apical du gradient, dans les blastulas et gastrulas

d'Astérie (Child, 1915 *c*). Les différences de coloration disparaissent quand les larves ont été préalablement tuées.

(*c*) *Différences de potentiel électrique.* L'idée d'une relation entre la polarité et des phénomènes électriques est déjà ancienne. Déjà Mathews (1903) avait montré que chez divers Hydraires (*Tubularia, Pennaria, Campanularia*), les régions apicales du tronc sont électro-négatives par rapport aux parties basales. Morgan et Dimon (1904) avaient cherché chez le Lombric, sans obtenir d'ailleurs de résultats bien nets, une relation entre la polarité physiologique et les différences de potentiel observées entre les différents niveaux du corps.

L. H. Hyman (1918), une élève de Child, a pensé que les courants électriques qui se manifestent entre les parties d'un organisme devaient être en rapport avec des différences de métabolisme, et par conséquent avec l'existence d'un gradient physiologique. La démonstration de cette relation entre les phénomènes électriques et l'existence d'un gradient a été fournie par L. H. Hyman (1920) sur la Tubulaire, et par Hyman et Galigher (1921) pour des Annélides (*Lumbriculus, Nereis*): les parties dominantes sont électro-négatives par rapport aux parties inférieures du gradient. Enfin, le parallélisme entre les gradients métaboliques, les gradients électriques, et même, dans quelques cas, certains phénomènes de galvanotropisme, a été montré par Hyman et Bellamy (1922) pour des animaux très variés: Éponges, Hydraires, Hydroméduses, Cténophores, Turbellariés, Annélides, Têtards; et la mesure des différences de potentiel doit être ajoutée aux techniques qui permettent la mise en évidence des gradients physiologiques.

B. Application de la notion de gradient à des organismes variés.

L'existence de gradients physiologiques a pu être démontrée chez des animaux appartenant aux embranchements les plus variés, et même chez de nombreux végétaux inférieurs. Nous passerons en revue sommairement l'ensemble de ces travaux.

Algues. Les poils, unicellulaires ou pluricellulaires, de nombreuses Algues présentent un gradient de susceptibilité très net. La partie dominante du gradient correspond à la zone de croissance, c'est-à-dire à l'extrémité basilaire pour les poils de *Fucus*, à l'extrémité apicale pour ceux de *Chondrus* ou de *Ceramium* (Child, 1917 *a*).

Protozoaires. Child (1914 *c*) a montré l'existence d'un gradient physiologique chez divers Ciliés (*Stentor, Stylonychia, Vorticella, Carchesium, Paramoecium*), l'extrémité antérieure étant la partie dominante. Ce gradient est d'autant plus net que la différenciation morphologique est plus marquée le long de l'axe antéro-postérieur: il est plus net chez *Stentor* ou *Stylonychia* que chez *Paramoecium*. Child et E. Deviney (1925) ont étudié spécialement le gradient de *Paramoecium*, par les procédés les plus variés.

L. H. Hyman (1917) a montré, chez les Amibes, l'existence d'une polarisation temporaire, se traduisant par un gradient de susceptibilité, en relation avec les mouvements amiboïdes, la partie la plus susceptible étant l'extrémité distale du pseudopode, c'est-à-dire le pôle antérieur de la marche. Ce gradient se

manifesterait avant l'apparition du pseudopode, et l'élévation du métabolisme au pôle antérieur amènerait la liquéfaction de l'ectoplasme à laquelle est due, suivant la théorie récente, la formation du pseudopode.

Éponges. Chez les Éponges *Leucosolenia* et *Granthia* (L. H. Hyman et Bellamy, 1922 ; Hyman, 1925), la région de plus forte susceptibilité et de plus grande activité respiratoire serait au voisinage de l'oscule.

Hydrozoaires. Les Hydraires, aux divers stades de leur développement, et les Hydroméduses, ont été très étudiés par l'école de Child. Les faits relatifs à la polarité et à l'hétéromorphose chez la Tubulaire, étant donnée leur importance théorique méritent qu'on s'y arrête. Ils ont été interprétés par Child (1915 *b*) sur la base des gradients physiologiques.

1º. La formation d'un stolon à l'extrémité aborale d'un fragment de Tubulaire n'a lieu que si le métabolisme du fragment est assez élevé : elle ne se produit que dans les conditions naturelles, et, dans les conditions du laboratoire, pour des troncs exceptionnellement vigoureux ou placés dans de l'eau de mer diluée (Child, 1907 *a*), qui élève le taux du métabolisme. Le stolon posséderait en effet un gradient propre, dirigé en sens inverse du gradient du polype, mais moins marqué (correspondant à des différences de métabolisme plus faibles), et inhibé par l'influence du polype[1]. Il ne se différenciera donc un stolon que si le polype de l'extrémité orale a un métabolisme assez élevé pour faire sentir sa dominance sur le développement de l'extrémité aborale. D'ailleurs, l'extrémité du stolon, après un certain temps de croissance, sort de la limite de dominance du polype, et se différencie en un polype : tel est le mode naturel de bourgeonnement des polypes chez la Tubulaire.

2º. Dans les fragments de tronc d'une certaine longueur, placés dans les conditions du laboratoire, l'activité du polype oral en régénération n'est pas assez forte pour que sa dominance se fasse sentir à l'extrémité aborale, et cette extrémité, isolée physiologiquement, régénère un polype. La formation de ce polype aboral est retardée par le gradient préexistant dans le tronc, de sens opposé à son propre gradient.

3º. Dans les fragments plus petits (10 mm. à 2 mm.), l'extrémité aborale est dans la zone de dominance du polype oral, et la formation du polype aboral est inhibée (le métabolisme général est trop bas pour qu'il se différencie un stolon). Dans les morceaux encore plus petits, la différence entre les deux extrémités dépendant du gradient primitif n'est souvent pas assez forte pour imposer une polarité au fragment, et l'hétéromorphose se produit alors, pour la même cause que chez les Planaires (cf. IIe partie).

4º. On a vu (cf. Introduction) qu'on obtenait souvent, dans les très petits fragments, soit des polypes incomplets, réduits à leurs parties les plus distales (ce qui est une nouvelle preuve de l'indépendance et de l'auto-différenciation des parties distales dans la régénération), soit des polypes complets de taille réduite : ces derniers correspondraient à une inhibition du gradient due à la stimulation

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[1] Child (1923) a pu réaliser la transformation de polypes en stolons, indépendamment de tout contact avec une surface solide, par l'action de divers toxiques exerçant une influence déprimante sur le métabolisme. La transformation d'un polype en stolon ou d'un stolon en polype correspond à une inhibition ou à une accélération du métabolisme, sans renversement de la polarité.

du fragment; on a vu qu'ils étaient en effet plus fréquents dans les régions basales du tronc, qui sont les plus stimulées par la section.

Cténophores. L'existence d'un gradient physiologique a été démontrée chez les Cténophores *Mnemiopsis* (Child, 1917 c) et *Pleurobrachia* (L. H. Hyman et Bellamy, 1922): l'extrémité dominante du gradient est au pôle aboral.

Turbellariés. Les faits relatifs aux Planaires ont déjà été exposés.

Annélides. L. H. Hyman (1916) a appliqué, dans un très beau mémoire, la notion de gradient physiologique à la régénération et au bourgeonnement chez divers Oligochètes d'eau douce (*Aelosoma, Dero, Lumbriculus, Tubifex*). Disons seulement que, chez ces Annélides, il se superpose au gradient primaire, antéro-postérieur, un gradient secondaire, dirigé en sens inverse, correspondant à la région de croissance postérieure. Ce gradient secondaire, plus ou moins développé suivant les espèces, serait toujours sous la dominance du gradient primaire (comme le gradient du stolon des Hydraires).

Échinodermes. La polarité de l'œuf et des jeunes larves des Astéries (Child, 1915 c) et des Oursins (Child, 1916 b) serait en relation avec l'existence d'un gradient physiologique.

Vertébrés. Selon L. H. Hyman (1921), les embryons de Téléostéens (*Fundulus, Gadus*, etc.) possèdent un gradient axial antéro-postérieur auquel vient se superposer un gradient dirigé en sens inverse, correspondant, comme chez les Annélides, à la zone postérieure de croissance. Puis, au fur et à mesure que l'organisation se complique, de nouveaux gradients secondaires apparaissent: ainsi, le cœur, le cerveau, les vésicules optiques et auditives, présentent dès leur apparition une susceptibilité élevée. Ces faits trouvent une application fort intéressante dans l'interprétation des *formes tératologiques* (L. H. Hyman, 1921; Bellamy, 1919). Les anomalies obtenues expérimentalement porteraient toujours sur les régions de haute susceptibilité, et les monstruosités se répartiraient en deux groupes: les unes correspondraient à une inhibition, à un arrêt de développement de ces parties; les autres, au contraire, résulteraient de leur capacité d'acclimatation et de guérison plus grande, et seraient caractérisées par leur hypertrophie.

On voit que, chez tous les êtres vivants, la disposition des gradients physiologiques serait en rapport avec le plan d'organisation, de symétrie. Tous les êtres posséderaient un gradient primaire, antéro-postérieur (ou apico-basal), correspondant généralement au gradient primitif de l'œuf; mais, avec les progrès de l'organisation, il apparaîtrait des gradients secondaires, se superposant ou même se substituant au gradient primitif.

Ainsi, l'organisation d'une Planaire comprendrait, en sus du gradient principal antéro-postérieur, un gradient dorso-ventral, un gradient médio-latéral (Child, 1913 b): l'existence du gradient dorso-ventral est prouvé par la vitesse plus grande de la régénération sur la face ventrale; celle du gradient médio-latéral par les expériences de régénération à partir de sections longitudinales ou obliques.

L'apparition de nouveaux gradients au cours de l'ontogénèse nous est montrée par le développement des Hydraires (Child, 1925): le gradient antéro-postérieur

de l'œuf subsiste jusque dans la *planula* nageuse; mais il apparaît à ce stade, par suite de l'isolement physiologique des parties postérieures dû à l'allongement de la *planula*, un nouveau gradient, postéro-antérieur, qui sera celui du premier polype, après la fixation de la *planula*. De même, le gradient des larves d'Échinodermes disparaîtrait à la métamorphose. Enfin, chez les animaux adultes, les gradients secondaires se multiplieraient et caractériseraient certains organes: nous avons déjà vu que les nerfs des Vertébrés possédaient un gradient propre (Tashiro, 1913 *b*; Child, 1914 *e*); il en serait de même de l'intestin, d'après les travaux d'Alvarez et de ses collaborateurs (Alvarez et Starkweather, 1918).

C. Variations du taux du métabolisme avec les conditions physiologiques.

Nous avons dit dans la IIe partie que le taux du métabolisme (ou l'âge physiologique) des tissus dépendait, non seulement de leur position dans l'organisme (gradient métabolique), mais aussi des conditions physiologiques, internes ou externes, auxquelles l'individu est soumis. Ces variations ne nous intéressent pas ici directement, mais nous devons rappeler brièvement les faits, car ils ont servi de base à des vérifications des idées de Child.

1º. L'*influence de l'âge* est bien connue: le taux du métabolisme, mesuré directement (cf. notamment L. H. Hyman, 1919 *d*), ou indiqué par la méthode de susceptibilité ou la méthode d'acclimatation (Child, 1913 *a*; etc.), est plus élevé chez les animaux jeunes (c'est-à-dire qu'il diminue, chez les Planaires, quand la taille augmente). La *reproduction sexuée*, la *reproduction asexuée*, la *régénération*, sont accompagnées d'un rajeunissement physiologique, en même temps que d'une réduction de taille.

2º. L'*inanition* provoquerait, pendant les premiers jours, un abaissement du métabolisme dans le tube digestif, mais l'inanition prolongée serait accompagnée, dans une seconde phase, d'une augmentation de métabolisme dans les tissus ectodermiques et le parenchyme (L. H. Hyman, 1919 *c*): l'inanition et la réduction de taille qu'elle détermine constitueraient un véritable rajeunissement physiologique (Child, 1914 *d*). La mort par inanition serait due à l'abolition du gradient métabolique.

3º. Les *traumatismes* exercent, comme nous l'avons vu, une influence accélératrice sur le métabolisme (stimulation).

4º. *Température.* E. H. Behre (1918), une élève de Child, a étudié l'influence des variations de température sur le métabolisme de *Planaria dorotocephala*. Un abaissement de la température produirait d'abord une diminution des oxydations, bientôt suivie (après 12 heures, environ)· d'une augmentation lente, que l'auteur considère comme un phénomène d'"acclimatation." Une élévation de température provoquerait des variations inverses. Il en résulte que le métabolisme d'une Planaire dépendrait non seulement de la température actuelle, mais des températures auxquelles elle a été précédemment soumise.

5º. Les *anesthésiques* et les *toxiques* (KCN) exercent sur le métabolisme une action inhibitrice. Mais, si les concentrations et les temps d'action utilisés ne sont pas mortels, il intervient des phénomènes d'"acclimatation," qui sont d'autant

plus rapides que le métabolisme était plus élevé: c'est le principe de la méthode d'acclimatation.

On a vu comment l'influence exercée par ces divers facteurs, internes ou externes, sur la fréquence de la tête ou la fréquence des scissions chez *Planaria dorotocephala* pouvait être expliquée par leur action sur le taux du métabolisme.

L'action des facteurs externes sur le métabolisme permet également, comme on l'a vu, l'interprétation des formes tératologiques dans le développement des Vertébrés, ou des transformations d'organes (transformation de polypes en stolons chez les Hydraires; Child, 1923). Enfin, divers agents inhibiteurs des oxydations, utilisés à faible concentration, peuvent produire (par inhibition différentielle) l'abolition ou même le renversement du gradient. Le gradient renversé se manifestera alors par la susceptibilité différentielle à un autre agent, ou au même agent, employé cette fois à dose mortelle. Child (1917 *a* et *b*) a trouvé chez les Algues des exemples de tels renversements du gradient.

A cette question de l'influence des facteurs externes sur le métabolisme et sur la régénération se rattachent les travaux tout récents d'un auteur américain, E. J. Lund (1921–26), relatifs à l'influence des courants électriques sur la régénération des polypes de l'Hydraire *Obelia commissuralis*. Lund non seulement a mis en évidence l'existence de différences de potentiel et la production de courants en rapport avec la polarité morphologique, mais il a pu mesurer l'intensité de ces courants inhérents à l'organisme polarisé, et montrer que l'application expérimentale de courants de même voltage (moins de 1 millivolt) à l'organisme (fragments de tiges ou entre-nœuds d'*Obelia*) peut influencer, inhiber ou inverser la polarité dans la régénération.

Lund conclut d'ailleurs de ses expériences que la polarité est fondamentalement de nature électrique: il pense, contrairement à l'école de Child, que les différences de potentiel observées entre les parties de l'organisme n'ont aucune relation nécessaire avec des différences dans l'intensité du métabolisme, et il suppose que c'est par la voie des courants électriques existant normalement dans un organisme polarisé que s'exercent directement les corrélations (Lund, 1925). Malgré cette différence d'interprétation, les travaux de Lund entrent naturellement dans le cadre de l'œuvre expérimentale de Child.

Un autre point des conclusions de Lund mérite d'être retenu ici. La polarité se manifeste chez *Obelia*, comme chez la Tubulaire, par des différences dans le temps d'apparition des polypes. Or, dans les expériences de Lund, les courants électriques n'influent pas sur la vitesse de croissance des polypes, mais ne font que retarder le début de la croissance. Dans la régénération normale, la croissance ne débute pas immédiatement après la section, mais elle est précédée par un certain délai, sorte de temps de latence. Lund prétend d'ailleurs que les différences observées dans le temps d'apparition normale des polypes (oraux ou aboraux) aux différents niveaux ne tiennent qu'à des variations de la durée de cette phase préliminaire, et non à des variations de la vitesse de croissance proprement dite.

Cette conclusion a été contestée (cf. L. H. Hyman, 1926)[1], mais il n'en est pas moins vrai que l'influence des courants électriques sur la polarité s'exerce pendant la phase préliminaire de la régénération (que leur passage a d'ailleurs pour effet de prolonger).

Cette conclusion de Lund doit être rapprochée, à mon avis, du fait que, dans les expériences de Child, les agents externes ont une influence effective sur la fréquence de la tête lorsqu'ils agissent pendant la période de "détermination de la tête," qui constitue la phase préliminaire de la régénération.

CONCLUSIONS DE LA IIIe PARTIE.

La notion de gradient est basée sur les réactions différentielles des parties d'un organisme polarisé à des influences physiologiques très diverses. La non-spécificité de l'action des agents employés indique bien qu'il s'agit de propriétés intrinsèques de l'organisme, de véritables *manifestations physiologiques de la polarité.* Ces différences entre les parties se ramèneraient d'ailleurs aux variations d'une seule variable, le taux du métabolisme.

L'originalité de la conception de Child consiste à rapprocher ces manifestations physiologiques de la polarité des manifestations morphologiques observées dans les phénomènes de régénération, et à établir une relation entre elles: l'expérience montre en effet une influence sur les modalités de la régénération des facteurs physiologiques généraux qui agissent sur le métabolisme. L'interprétation de ces expériences a conduit Child à imaginer certains schémas, qui peuvent paraître un peu arbitraires, ou que leur trop grande souplesse rend peut-être suspects, mais il ne faut pas oublier qu'ils constituent la seule tentative qui ait été faite pour atteindre le déterminisme des faits de régénération par une voie purement physiologique.

En tous cas, l'existence des manifestations physiologiques de la polarité, la stimulation des fragments consécutive à la section, la différenciation physiologique précédant la scission dans la reproduction asexuée, l'influence des anesthésiques et des toxiques sur la régénération, l'existence d'une période de sensibilité à cette action, et bien d'autres points de l'œuvre de Child, sont des faits positifs, indépendants de toute théorie.

RÉSUMÉ ET CONCLUSIONS GÉNÉRALES.

Les pages précédentes, qui résument de nombreux travaux datant de ces vingt dernières années, donneront, je pense, une idée des progrès qui ont été réalisés, depuis les spéculations de Bonnet et la théorie des substances formatives, sur la question de la polarité organique. Le problème de la polarité qui domine, comme on a pu le voir, tous les problèmes de la régénération, au moins chez les animaux inférieurs, est actuellement abordé par la voie expérimentale, et tout un ensemble de résultats positifs sont dès maintenant acquis.

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[1] Il semble bien que, dans beaucoup des cas énumérés dans l'Introduction, il existe des différences polaires dans la vitesse de la croissance proprement dite.

Tout en renvoyant aux conclusions partielles des différents chapitres, je reviendrai ici sur quelques points importants, relatifs à la définition, à la nature, à l'origine et aux modifications de la polarité.

1°. *Définition de la polarité*. On a vu dans l'Introduction de cet article comment se manifeste la polarité dans les phénomènes de régénération : (*a*) la *régénération polaire* consiste en la régénération d'une structure antérieure à l'extrémité antérieure d'un fragment, et la régénération d'une structure postérieure à l'extrémité postérieure (*manifestations qualitatives de la polarité*). Mais cette loi souffre de nombreuses exceptions (cas d'*hétéromorphose*), et Child a d'ailleurs montré que certains cas de régénération constituaient des intermédiaires entre la régénération polaire et l'hétéromorphose ; (*b*) une étude expérimentale plus précise, quantitative, de la régénération, montre, à divers points de vue, des différences de comportement entre les fragments provenant de niveaux différents de l'organisme : ces faits constituent ce que nous avons appelé les *manifestations quantitatives* de la polarité ; (*c*) enfin, Child a ajouté à ces données du domaine de la régénération un ensemble de faits qui indiquent une variation continue des propriétés physiologiques des tissus, parallèle à la polarité morphologique ("gradient physiologique"; *manifestations physiologiques de la polarité*). Cette définition physiologique de la polarité est, comme on l'a vu, la plus générale, et on doit par conséquent la considérer comme la meilleure.

2°. *Nature de la polarité*. Une théorie de la polarité doit permettre d'en synthétiser toutes les manifestations (qualitatives, quantitatives et physiologiques). Seule la théorie de Child semble remplir ces conditions.

On a vu en effet que la *théorie des substances formatives* sous sa forme actuelle ne pouvait rendre compte que des phénomènes qualitatifs de la polarité. Si cette théorie semble permettre une interprétation satisfaisante des faits de régénération chez les végétaux (travaux de J. Loeb sur *Bryophyllum*) où les manifestations quantitatives de la polarité seraient inexistantes, on a vu qu'elle s'était montrée insuffisante dans le cas des animaux (travaux de Loeb sur la Tubulaire).

Quant à la théorie de Child, son point central est l'hypothèse d'une gradation continue dans l'intensité du métabolisme protoplasmique, dans l'"âge physiologique" des tissus, d'une extrémité à l'autre de l'organisme polarisé (*gradient métabolique*). De cette hypothèse fondamentale découle directement l'interprétation des manifestations physiologiques de la polarité. Quant aux manifestations (qualitatives et quantitatives) de la polarité dans les phénomènes de régénération, Child a imaginé quelques schémas physiologiques simples qui permettent de les rattacher à la notion de gradient métabolique.

L'hypothèse du gradient métabolique n'est pas arbitraire : elle a été vérifiée, non seulement indirectement, par les conséquences qui en découlent, mais encore directement, par des mesures d'intensité respiratoire. D'ailleurs, même si l'on se refuse à accepter la notion d'une gradation de métabolisme, il semble difficile d'interpréter les manifestations quantitatives et les manifestations physiologiques de la polarité autrement qu'en admettant une gradation continue de "quelque chose," d'une propriété inhérente à l'organisme, le long de l'axe morphologique.

3⁰. *Origine et modifications de la polarité.* La polarité n'est pas une propriété fixe, une qualité indélébile des organismes qui la possèdent; elle est au contraire labile, elle peut être inhibée ou renversée sous des influences externes très variées. J. Loeb (cf. Introduction) a le premier attiré l'attention sur l'importance des facteurs externes dans la régénération polaire et l'hétéromorphose. Child et Lund ont étudié méthodiquement l'action de différents facteurs externes sur la polarité.

Ces faits paraissent difficiles à concevoir dans l'hypothèse des substances organo-formatives, tandis que la notion de gradient métabolique a permis à Child de les interpréter très aisément: l'intensité du métabolisme est en effet sous la dépendance étroite des facteurs physiologiques, aussi bien internes (âge, état de nutrition) qu'externes (température, action des anesthésiques, des toxiques et, peut-être aussi, des courants électriques, etc.). Rappelons seulement ici que les facteurs externes ont toujours une action différentielle sur les divers échelons du gradient préexistant, les régions à métabolisme élevé étant à la fois les plus susceptibles et les plus rapidement "acclimatées."

Les facteurs externes ont une influence décisive sur la régénération lorsqu'ils agissent pendant les premières heures qui suivent la section, c'est-à-dire pendant la phase de latence qui précède le début de la croissance (Lund, Child). La polarité se trouve donc déterminée, dans la régénération, au cours de cette phase préliminaire, de durée relativement très courte.

Dans l'ontogénèse, la polarité primitive de l'organisme aurait pour origine, suivant Child, une influence étrangère à l'organisme: les gradients axiaux ne seraient qu'une réponse à un stimulus extérieur lui-même localisé et polarisé.

Les théories de Child semblent donc être seules actuellement à fournir une interprétation d'ensemble des faits relatifs à la polarité organique. Elles nous permettent d'atteindre le déterminisme des faits de régénération et de régulation, si complexes chez les Invertébrés inférieurs, et nous délivrent de la "force régulatrice," de l'"entéléchie" de Driesch, dernière incarnation de la "force vitale." Si Child fait parfois intervenir les propriétés d'"irritabilité" du protoplasme, il n'y a pas de doute que ces mécanismes ne soient réductibles en dernière analyse à des forces physico-chimiques, et il vaut mieux s'en tenir provisoirement à ces notions que de recourir à des schémas physico-chimiques simplistes ou à des substances formatives spécifiques, mais imaginaires.

Pourtant, il est clair que la question de la régénération est loin d'avoir atteint le terme final de son évolution. La théorie de Child, avec son caractère un peu schématique, formel, étroit même, ne peut nous satisfaire encore pleinement, pas plus que la théorie chromosomique de T. H. Morgan ne nous fournit une conception qui semble devoir être définitive des phénomènes de l'hérédité. La théorie de Child et la théorie chromosomique de l'hérédité, très différentes par leur objet et même opposées dans leur esprit, sont très comparables à divers points de vue, par le nombre et le caractère au premier abord arbitraire des hypothèses sur lesquelles elles reposent, par la quantité de faits, d'observations et d'expériences qu'elles permettent de synthétiser, par le nombre et la variété des vérifications et

des confirmations qu'elles ont recues; et on peut dire que le problème de la régénération chez les animaux inférieurs a été porté par les recherches de Child à un stade très comparable à celui qu'a atteint la question de l'hérédité après les travaux de T. H. Morgan.

INDEX BIBLIOGRAPHIQUE.

ALLMAN, G. J. (1864). *Report of the British Association for the Advancement of Science.*

ALVAREZ, W. C. et E. STARKWEATHER (1918). "The metabolic gradient underlying intestinal peristalsis." *Amer. Journ. Physiol.* **46**.

BEHRE, E. H. (1918). "An experimental study of acclimatisation to temperature in *Planaria dorotocephala*." *Biol. Bull.* **35**.

BELLAMY, A. W. (1919). "Differential susceptibility as a basis for modification and control of early development in the frog." *Biol. Bull.* **37**.

BICKFORD, E. E. (1894). "Notes on regeneration and heteromorphosis in Tubularian Hydroids." *Journ. Morph.* **9**.

BILLARD, A. (1904). "Contribution à l'étude des Hydroïdes." *Ann. Sc. Nat.* s. 8, **20**.

BOHN, G. (1921). *La forme et le mouvement.* Paris.

BONNET, G. (1745). *Traité d'insectologie.* Seconde partie, Paris.

BUCHANAN, J. W. (1922). "The control of head formation in *Planaria* by means of anaesthetics." *Journ. of Exp. Zool.* **36**.

CHILD, C. M. (1903). "Form regulation in *Cerianthus*. II. The effect of position, size and other factors upon regeneration." *Biol. Bull.* **6**.

—— (1906 *a*). "The relation between regulation and fission in *Planaria*." *Biol. Bull.* **11**.

—— (1906 *b*). "The relation between functional regulation and form regulation." *Journ. of Exp. Zool.* **3**.

—— (1907 *a*). "An analysis of form-regulation in *Tubularia*. I. Stolon formation and polarity." *Arch. f. Entwmech.* **23**.

—— (1907 *b*). "An analysis of form-regulation in *Tubularia*. III. Regional and polar differences in the relation between primordium and hydrant." *Arch. f. Entwmech.* **23**.

—— (1907 *c*). "An analysis of form-regulation in *Tubularia*. V. Regulation in short pieces." *Arch. f. Entwmech.* **24**.

—— (1910). "Physiological isolation of parts and fission in *Planaria*." *Arch. f. Entwmech.* **30**.

—— (1911 *a*). "Experimental control of morphogenesis in the regulation of *Planaria*." *Biol. Bull.* **20**.

—— (1911 *b*). "Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. I. The axial gradient in *Planaria dorotocephala* as a limiting factor in regulation." *Journ. of Exp. Zool.* **10**.

—— (1913 *a*). "Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. V. The relation between resistance to depressing agents and rate of metabolism in *Planaria dorotocephala* and its value as a method of investigation." *Journ. of Exp. Zool.* **14**.

—— (1913 *b*). "Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. VI. The nature of the axial gradients in *Planaria* and their relation to antero-posterior dominance, polarity and symmetry." *Arch. f. Entwmech.* **37**.

—— (1914 *a*). "Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. VII. The stimulation of pieces by section in *Planaria dorotocephala*." *Journ. of Exp. Zool.* **16**.

—— (1914 *b*). "Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. VIII. Dynamic factors in head-determination in *Planaria*." *Journ. of Exp. Zool.* **17**.

—— (1914 *c*). "The axial gradient in ciliate Infusoria." *Biol. Bull.* **26**.

—— (1914 *d*). "Starvation, rejuvenescence and acclimatation in *Planaria dorotocephala*." *Arch. f. Entwmech.* **38**.

—— (1914 *e*). "Susceptibility gradients in animals." *Science*, **39**.

—— (1915 *a*). *Senescence and Rejuvenescence.* Chicago, 1915.

—— (1915 *b*). *Individuality in Organisms.* Chicago, 1915.

—— (1915 *c*). "Axial gradients in the early development of the starfish." *Amer. Journ. Physiol.* **37**.

—— (1916 *a*). "Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. IX. The control of head-form and head-frequency in *Planaria* by means of potassium cyanide." *Journ. of Exp. Zool.* **21**.

—— (1916 *b*). "Axial susceptibility gradients in the early development of the sea urchin." *Biol. Bull.* **30**.

CHILD, C. M. (1917 a). "Susceptibility gradients in the hairs of certain marine Algae." *Biol. Bull.* **32**.

—— (1917 b). "Experimental alteration of the axial gradient in the Alga *Griffithsia bornetiana*." *Biol. Bull.* **32**.

—— (1917 c). "The gradient in susceptibility to cyanides in the meridional conducting path of the Ctenophore *Mnemiopsis*." *Amer. Journ. Physiol.* **43**.

—— (1919). "Demonstration of the axial gradients by means of potassium permanganate." *Biol. Bull.* **36**.

—— (1920 a). "Some considerations concerning the nature and origin of physiological gradients." *Biol. Bull.* **39**.

—— (1920 b). "Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. X. Head-frequency in *Planaria dorotocephala* in relation to age, nutrition and motor activity." *Journ. of Exp. Biol.* **30**.

—— (1921). "The axial gradients in Hydrozoa. IV. Axial gradations in rate and amount of reduction of potassium permanganate in various Hydroids and Medusae." *Biol. Bull.* **41**.

—— (1923). "The axial gradients in Hydrozoa. V. Experimental axial transformations in Hydroids." *Biol. Bull.* **45**.

—— (1925). "The axial gradients in Hydrozoa. VI. Embryonic development of Hydroids." *Biol. Bull.* **48**.

CHILD, C. M. et DEVINEY, E. (1925). "Contributions to the physiology of *Paramecium caudatum*." *Journ. of Exp. Zool.* **43**.

CHILD, C. M. et HYMAN, L. H. (1919). "The axial gradients in Hydrozoa. I. *Hydra*." *Biol. Bull.* **36**.

DEHORNE, L. (1916). *Les Naïdimorphes et leur reproduction asexuée*. Thèse. Paris.

DRIESCH (1897). "Studien über das Regulationsvermögen der Organismen. I. Von den regulativen Wachsthums- und Differenzirungsfähigkeiten der *Tubularia*." *Arch. f. Entwmech.* **5**.

—— (1899). "Studien über das Regulationsvermögen der Organismen. II. Quantitative Regulationen bei der Reparation der *Tubularia*." *Arch. f. Entwmech.* **9**.

DRZEWINA, A. et BOHN, G. (1912). "Résistance de divers animaux marins à la privation d'oxygène." *C.R. Soc. Biol.* **73**.

—— —— (1913). "Anoxybiose et polarité chimique." *C.R. Acad. Sc.* **156**.

—— —— (1916). "Phénomènes de réduction et d'activation chez les Hydres à la suite de variations de la teneur de l'eau en oxygène." *C.R. Soc. Biol.* **79**.

DURBIN, M. L. (1909). "An analysis of the rate of regeneration throughout the regenerative process." *Journ. of Exp. Zool.* **7**.

ELLIS, M. M. (1909). "The relation of the amount of tail regenerated to the amount removed in tadpoles of *Rana clamitans*." *Journ. of Exp. Zool.* **7**.

GAST, R. et GODLEWSKI, E. (1903). "Die Regulationserscheinungen bei *Pennaria cavolinii*." *Arch. f. Entwmech.* **16**.

GOEBEL, K. (1908). *Einleitung in die experimentelle Morphologie der Pflanzen*.

GOLDFARB, A. J. (1907). "Factors in regeneration of a compound Hydroid *Endendrium ramosum*." *Journ. of Exp. Zool.* **4**.

HINRICHS, M. A. (1924 a). "A study of the physiological effects of caffein upon *Planaria dorotocephala*." *Journ. of Exp. Zool.* **40**.

—— (1924 b). "A demonstration of the axial gradient by means of photolysis." *Journ. of Exp. Zool.* **41**.

HYMAN, L. H. (1916). "An analysis of the process of regeneration in certain microdrilous Oligochaetes." *Journ. of Exp. Zool.* **20**.

—— (1917). "Metabolic gradients in *Amoeba* and their relation to the mechanism of amoeboid movement." *Journ. of Exp. Zool.* **24**.

—— (1918). "Suggestions regarding the causes of bioelectric phenomena." *Science*, **48**.

—— (1919 a). "On the action of certain substances on oxygen consumption. II. Action of potassium cyanide on *Planaria*." *Amer. Journ. Physiol.* **48**.

—— (1919 b). "On the action of certain substances on oxygen consumption. III. Action of potassium cyanide on some Coelenterates and Annelids." *Biol. Bull.* **37**.

—— (1919 c). "Physiological studies on *Planaria*. I. Oxygen consumption in relation to feeding and starvation." *Amer. Journ. Physiol.* **49**.

—— (1919 d). "Physiological Studies on *Planaria*. III. Oxygen consumption in relation to age (size) differences." *Biol. Bull.* **37**.

—— (1920). "The axial gradients in Hydrozoa. III. Experiments on the gradient of *Tubularia*." *Biol. Bull.* **38**.

—— (1921). "The metabolic gradients of vertebrate embryos. I. Teleost embryos." *Biol. Bull.* **40**.

—— (1923). "Physiological studies on *Planaria*. V. Oxygen consumption of pieces with respect to length, level, and time after section." *Journ. of Exp. Zool.* **37**.

—— (1925). "Respiratory differences along the axis of the sponge *Granthia*." *Biol. Bull.* **48**.

HYMAN, L. H. (1926). "The axial gradients in Hydrozoa. VIII. Respiratory differences along the axis in *Tubularia* with some remarks on regeneration rate." *Biol. Bull.* **50**.

HYMAN, L. H. et BELLAMY, A. W. (1922). "Studies on the correlation between metabolic gradients, electrical gradients, and galvanotaxis. I." *Biol. Bull.* **43**.

HYMAN, L. H. et GALIGHER, A. E. (1921). "Direct demonstration of the existence of a metabolic gradient in Annelids." *Journ. of Exp. Zool.* **34**.

KING, H. D. (1900). "Further studies on regeneration in *Asterias vulgaris.*" *Arch. f. Entwmech.* **9**.

KNIEP, H. (1907). "Beiträge zur Keimungsphysiologie und Biologie von *Fucus.*" *Jahrb. f. wiss. Bot.* **44**.

LOEB, J. (1905). *Studies in general physiology.* Chicago.

—— (1906). *The dynamics of living matter.* New York.

—— (1916). *The organism as a whole.* New York.

—— (1926). *Les bases physico-chimiques de la régénération.* (Traduit de l'anglais par H. Mouton.) Paris.

LUND, B. L. (1918). "The toxic action of KCN and its relation to the state of nutrition and age of the cell as shown by *Paramecium* and *Didinium.*" *Biol. Bull.* **35**.

LUND, E. J. (1917). "The rate of intracellular oxidation in *Paramecium caudatum* and its relation to the toxic action of KCN." *Anat. Rec.* **14**, *Proc. Am. Soc. Zool.*

—— (1918 a), "Quantitative studies of intracellular respiration. II. The rate of oxidations in *Paramecium caudatum* and its independence of the toxic action of KCN." *Amer. Journ. Physiol.* **45**.

—— (1918 b). "Quantitative studies of intracellular respiration. III. Relation of the state of nutrition of *Paramecium* to the rate of intracellular oxidation." *Amer. Journ. Physiol.* **47**.

—— (1921). "Experimental control of organic polarity by the electric current. I. Effects of the electric current on regenerating internodes of *Obelia commissuralis.*" *Journ. of Exp. Zool.* **34**.

—— (1922). "Experimental control of organic polarity by the electric current. II. The normal electrical polarity in *Obelia.* A proof of its existence." *Journ. of Exp. Zool.* **36**.

—— (1923). "Experimental control of organic polarity by the electric current. III. Normal and experimental delay in the initiation of polypformation in *Obelia* internodes." *Journ. of Exp. Zool.* **37**.

—— (1924). "Experimental control of organic polarity by the electric current. IV. The quantitative relations between current density, orientation and inhibition of regeneration." *Journ. of Exp. Zool.* **39**.

—— (1925). "Experimental control of organic polarity by the electric current. V. The nature of the control of organic polarity by the electric current." *Journ. of Exp. Zool.* **41**.

—— (1926). "The electrical polarity of *Obelia* and frog's skin and its reversible inhibition by cyanide, ether, and chloroform." *Journ. of Exp. Zool.* **44**.

MATHEWS, A. P. (1903). "Electrical polarity in the Hydroids." *Amer. Journ. of Physiol.* **8**.

MORGAN, T. H. (1900). "Regeneration: old and new interpretations." *Biol. Lectures from the Mar. Biol. Labor. of Woods Holl,* 1899.

—— (1901). "Regeneration in *Tubularia.*" *Arch. f. Entwmech.* **11**.

—— (1904). "An analysis of the phenomena of organic 'polarity.'" *Science,* N.S. **20**.

—— (1905). "'Polarity' considered as a phenomenon of gradation of materials." *Journ. of Exp. Zool.* **2**.

—— (1906). "The physiology of regeneration." *Journ. of Exp. Zool.* **3**.

—— (1908). "Some further records concerning the physiology of regeneration in *Tubularia.*" *Biol. Bull.* **14**.

MORGAN, T. H. et DIMON, A. C. (1904). "An examination of the problems of physiological 'polarity' and of electrical polarity in the earthworm." *Journ. of Exp. Zool.* **1**.

MORGAN, T. H. et STEVENS, N. M. (1904). "Experiments on polarity in *Tubularia.*" *Journ. of Exp. Zool.* **1**.

MORGULIS, S. (1907). "Observations and experiments on regeneration in *Lumbriculus.*" *Journ. of Exp. Zool.* **4**.

—— (1909). "Contributions to the physiology of regeneration. I. Experiments on *Podarke obscura.*" *Journ. of Exp. Zool.* **7**.

ROBBINS, H. S. et CHILD, C. M. (1920). "Carbon dioxide production in relation to regeneration in *Planaria dorotocephala.*" *Biol. Bull.* **38**.

SACHS, J. (1892). *Stoff und Form der Pflanzenorgane. Gesamm. Abhandl. über Pflanzenphysiol.* **2**. Leipzig.

STAHL, E. (1885). "Über den Einfluss der Beleuchtungsrichtung auf die Teilung der Equisetumsporen." *Berichte deutsch. Bot. Ges.* **3**.

STOCKARD, CH. R. (1907). "Studies on tissue growth. I. An experimental study of the rate of regeneration in *Cassiopea xamachana.*" *Carneg. Instit. of Washington, Public. No.* 103, **61**.

TASHIRO, S. (1913 a). "A new method and apparatus for the estimation of exceedingly minute quantities of carbon dioxide." *Amer. Journ. Physiol.* **32.**

—— (1913 b). "Carbon dioxide production from nerve fibres when resting and when stimulated; a contribution to the chemical basis of irritability." *Amer. Journ. Physiol.* **32.**

TORREY (1910). "Biological studies on *Corymorpha*. IV. Budding and fission in heteromorphic pieces and the control of polarity." *Biol. Bull.* **19.**

VANDEL, A. (1921). "Recherches expérimentales sur les modes de reproduction des Planaires triclades paludicoles." *Bull. Biol. France et Belgique,* **55.**

WINKLER, H. (1900). "Über den Einfluss äusserer Faktoren auf die Teilung des Eies von *Cystoma barbata.*" *Berichte deutsch. Bot. Ges.* **18.**

WINTREBERT, P. (1921). "La contraction rythmée aneurale des myotomes chez les embryons de Sélaciens." *Arch. de Zool. exp. et gén.* **60.**

ZELENY, CH. (1917). "The effect of degree of injury, level cut and time within the regenerative cycle upon the rate of regeneration." *Proc. Nat. Acad. Sc.* **3.**

THE CONDITIONS GOVERNING PARTURITION

By F. H. A. MARSHALL.

"WE may be said to be in the dark," wrote Michael Foster, "as to why the uterus, after remaining for months subject to futile contractions, is suddenly thrown into powerful and efficient action, and within, it may be, a few hours or even less, gets rid of the burden which it has borne with such tolerance for so long a time. None of the hypotheses which have been put forward can be considered as satisfactory." Williams has arranged these hypotheses under twelve heads and has briefly discussed each of them. He concludes by saying that most of them are extremely unsatisfactory and none can be of universal application.

It is the purpose of this article to give a connected account of certain recent investigations which seem to throw light upon the problem. In so doing it may be well to state at the outset that on the view here adopted the culminating act whereby pregnancy is brought to an end is the result, not of one or two factors, as so many of the hypotheses which have been put forward imply, but of a combination of conditions, all of which contribute to the end in question, but some of which, though normally participating, are not necessarily essential to its fulfilment. There is another criticism which may be made here though it is almost equally applicable to the usual physiological mode of regarding any of the bodily mechanisms; I refer to the tendency to regard these as being normally complete and perfect and having a definite end or purpose for which the mechanism exists. This tendency takes no account of the theory of evolutionary development to which all biologists now subscribe. It is, on the other hand, a teleological mode of thought which is specially characteristic of physiology and dates back to the time of the universal acceptance of the theory of specific design. It has been justly remarked that the Darwinian theory has had less influence on physiological study than on any other division of biology, for physiologists are still wont to regard the function of an organ or the mechanism of an organic structure as though it had come into being completely adjusted to fulfil the part which it normally plays. Yet in the light of evolutionary knowledge it is obvious that the functional correlations which exist between the different organs of the body (such, for example, as the ovaries, uterus and mammary glands) must have come into existence gradually, and that there have been stages in phylogenetic history when these were imperfect or unessential—while at the same time sufficiently developed to be advantageous—for the normal working of the body. These considerations have a definite bearing on the present problem, and it would seem likely that the study of comparative physiology will show that the factors concerned in parturition may vary in the different groups of mammals and that those which are usually regarded as higher in the class will show a greater degree of physiological perfection in the act of giving birth than is to be found in the lower orders. Moreover, it is not improbable that

there are variations in the mechanism at work even within the limits of a single order, for otherwise it is difficult to explain such facts as that the rabbit has a period of pregnancy of about thirty days and brings forth its young naked and helpless whereas the smaller guinea-pig carries its young sixty-three days and produces them in such a condition that they are able to fend for themselves.

The ovaries and pseudo-pregnancy.

Of the recent investigations relating to the problem as to the causes of parturition, those upon the condition known as pseudo-pregnancy are among the most suggestive. The species in which pseudo-pregnancy is known to occur are the marsupial cat (Hill and O'Donoghue), the opossum (Hartman), and the dog (Marshall and Halnan). To these must be added the rabbit under experimental conditions, as after a sterile copulation (Ancel and Bouin, Hammond and Marshall), and the cat, also abnormally under similar conditions to the rabbit (Doncaster). In these animals during pseudo-pregnancy the corpus luteum persists in the same kind of way as during gestation, and the uterus and mammary glands undergo hypertrophy in correlation with the growth and persistence of the corpus luteum. Indeed, the evidence points clearly to the conclusion that pseudo-pregnancy is dependent upon the corpus luteum. Thus, in the rabbit, which normally ovulates only after copulation, pseudo-pregnancy can be brought about by allowing the doe to undergo sterile coition (as with a vasectomised buck, or after the doe herself had been rendered sterile by severing the Fallopian tubes). The corpus luteum after a period of functional activity eventually undergoes regressive changes and in association with these the uterus also regresses and the mammary glands secrete milk in much the same way as they do at the end of true pregnancy. In the marsupial cat, the opossum and the bitch the same sequence of events occurs, but in these the processes are spontaneously initiated (without coition) by ovulation during oestrus which is followed by the development of the corpora lutea. Many instances are known of bitches which have produced sufficient milk at the end of pseudo-pregnancy to admit of their suckling another bitch's pups, and similarly the marsupial cat and the opossum are known freely to lactate under the same conditions. Moreover at a stage of involution which may be considered as marking the close of pseudo-pregnancy the animals commonly display instincts or habits which are normally associated with parturition. Thus the bitch will prepare a bed as if for a litter of pups, the marsupial cat cleans out her pouch as though for the reception of young, and the doe rabbit plucks her breast of fur which she uses to line a nest. Since these habits are displayed at the end of pseudo-pregnancy, which, as stated above, is dependent upon the persistence of the corpus luteum, it is not unreasonable to suppose that the processes associated with actual parturition after true pregnancy are correlated similarly with ovarian changes depending upon the involution of the corpus luteum and not solely upon the presence of the developed foetuses and the hypertrophied uterine muscles. These considerations clearly suggest that one of the essential conditions for the occurrence of parturition is a certain phase of the ovarian cycle.

Ancel and Bouin have described a gland lying between the uterine muscles and the mucosa—the myometrial gland—and they regard the tolerance of the uterus to the foetus in the latter part of pregnancy as due to this gland. They take the view that in the earlier part of pregnancy the corpus luteum exercises this function. Other observers, however, have failed to confirm the uniform presence of the myometrial gland. Moreover, Hammond has shown that the corpus luteum in the rabbit does not undergo retrogression until, at any rate, the very end of pregnancy, and there seems every reason to regard it as being functional throughout the whole, or practically the whole, of the period.

There is no experimental evidence, however, that the ovary acts directly either in promoting or in inhibiting uterine contraction. Observations on the effects of ovarian extract upon the isolated uterus suspended in Ringer's or Locke's solutions show results in no way differing from those of other tissue extracts. Itagaki has stated that extract of corpus luteum produces an increase in tone in the uterine muscle, but that it sometimes has the opposite effect. Liquor folliculi also has been found to cause increase in tone both on the uterus and other muscles. It is not clear, however, that the augmentor effect when present is not due to the serum constituent or to histamine-like bodies. The conclusion may probably be drawn that the ovary does not possess any direct specific influence upon the contractility of the uterine muscle.

The pituitary.

It is now well recognised that the secretion of the pituitary gland has a specific effect on uterine muscle. Extract of the posterior lobe is widely used by obstetricians to expedite parturition, especially in cases of difficulty, but owing to its powerful action in producing the contraction of the muscles it is necessarily employed with great caution. The specific influence of the extract has been taken advantage of for purposes of standardising its strength, and in doing this the virgin uterus of the guinea-pig is used (Burn and Dale). In view of these considerations Blair Bell and others have suggested that the posterior lobe may be the source of the stimulus for labour. If this is so the question remains as to why the stimulation from the pituitary should be especially great at this time.

Certain experiments carried out by Dixon and extended by Dixon and the present writer have suggested a partial solution of this question. In the earlier of these Dixon obtained evidence that commercial ovarian extract had an excitatory influence on the posterior lobe, as manifested by the effect produced by the secretion of an activating substance, believed to be derived from the posterior lobe, into the cerebro-spinal canal. In all the experiments, samples of cerebro-spinal fluid were taken at short intervals from an anaesthetised dog and tested upon a virgin guinea-pig's uterus suspended in Ringer's fluid. The extract to be tested was injected into the circulation of the dog. If the result was positive it was held to indicate that the extract employed had had a stimulating effect on the posterior lobe and caused secretion from that gland. The tissue extracts used included testis, epididymis, pancreas, ovary, and corpus luteum, besides some others, and with all of these except ovary the results were negative. Ovarian extract, on the other hand, was

found to have a positive result in that samples of cerebro-spinal fluid withdrawn from the injected dog were observed to promote the contraction of the guinea-pig's uterus.

Doubt has been expressed, more particularly by Abel, as to whether the secretions of the posterior lobe do actually pass into the cerebro-spinal fluid. Thus, Abel quotes the experiments of C. and M. Oehme, who state that with the normal unconcentrated cerebro-spinal fluid of laboratory animals they could not obtain any definite evidence of action on the guinea-pig's uterus, as well as those of Leschke whose results were similarly negative in this respect. On the other hand, Herring has demonstrated the passage of colloidal masses from the pars intermedia of the pituitary gland through the pars nervosa and ultimately into the third ventricle and thence into the cerebro-spinal fluid. Cushing and Goetsch as a result of their observations have independently come to a similar conclusion. Experimental evidence of the passage of pituitary secretion into the cerebro-spinal fluid has been adduced by Cow, and more recently by Trendelenburg, besides by Dixon as above quoted. Moreover Miura has found that the fluid has less or no effect on the uterus if the pituitary had been previously extirpated or its stalk divided. Lastly according to Mayer, human cerebro-spinal fluid obtained by lumbar puncture during parturition, when injected intravenously into another patient with deficient pains, may have a stimulating action on the uterus.

Experiments at different stages of the oestrous cycle.

In the experiments by Dixon and the present writer, extracts of sows' ovaries taken at different stages of pregnancy and of the oestrous cycle were employed. Everything was known as to the precise condition of the animals at the times of killing, and a fairly complete series was obtained. The method of experiment was the same as that previously adopted by Dixon and consisted of tapping the cerebro-spinal fluid of an anaesthetised dog and the subsequent injection of the ovarian extract into the femoral vein. The extract was made by pounding up the ovaries in a mortar with Ringer's solution and boiling and filtering.

Samples of the cerebro-spinal fluid were subsequently withdrawn at short intervals and tested for pituitary secretion on the virgin uterus of a guinea-pig in the usual way. Of five experiments with ovaries obtained during different stages of pregnancy and therefore containing fully formed and presumably active corpora lutea, all were negative, no stimulating action on the part of the cerebro-spinal fluid being detected. Of two experiments with ovaries about twelve days before parturition was due, one was negative and the other problematically positive. Four experiments with ovaries taken at or just before parturition when the corpora lutea were undergoing regression were emphatically positive, the uterus of the guinea-pig undergoing contractions equal to those produced by two drops of 1 per cent. pituitary extract. Further experiments were performed using ovaries of non-pregnant sows at different stages of the oestrous cycle, and, speaking generally, the results were negative if fully formed corpora lutea were present but positive in the absence of these bodies.

The experiments then supported the view that in the presence of fully formed and presumably functional corpora lutea the normal ovarian secretion is largely or entirely in abeyance, and this is the condition for a short time between the oestrous periods but more particularly during pregnancy; that is to say, the corpus luteum may be supposed so to dominate the ovarian metabolism at these times that the ovarian hormone which at other times activates the pituitary is inhibited or else is neutralised by the hormone of the corpus luteum. At the end of pregnancy when the corpus luteum is in a state of regression and no longer active the normal ovarian secretory activity is resumed and the pituitary is stimulated to secrete in greater quantity. When the threshold stimulus of the pituitary upon the uterus is reached and passed labour pains set in and parturition results. The growing irritability of the uterus in the final stages of pregnancy may be partly accounted for in this way (as being correlated with the involution of the corpus luteum), but it must not be supposed that the ovario-pituitary endocrine mechanism is the sole factor in parturition. There are certainly other necessary conditions, as will be made clear below.

Further experiments with ovarian and pituitary extracts.

Parkes and Bellerby have described experiments which to some extent confirm the views expressed above concerning the ovary and the pituitary as among the causes of parturition. They found that injection of ovarian extract in mice produced abortion during the latter stages of pregnancy. The extract may be interpreted as having a swamping or neutralising influence upon the secretion produced by the corpus luteum of pregnancy, or as acting directly upon the pituitary. Parkes' ovarian extracts, however, were dissolved in alcohol like his other oestrus-producing extracts and he found that the oestrus-producing hormone was insoluble in saline. It would appear, therefore, that the active substance used by Dixon and those employed by Parkes were not identical, but they probably contained certain of the same principles. Extract of corpus luteum did not produce abortion.

Knaus has called attention to the hypertrophy of the uterine muscle as an important factor in parturition. He quotes observations of Hammond's showing that in the rabbit the increase during the first sixteen days (about half) of pregnancy is slight, but rises rapidly from this time onwards continuing at an accelerated rate in the last few days. In correlation with these facts Knaus found that the effect of pituitary extract upon the uterus increased steadily as pregnancy advanced. During the first ten days the maximum contraction in each muscle fibre was unable to exert a sufficiently great mechanical effect to influence the transport of the embryo; during the next seven days it could not disturb the connexion between the uterine wall and the placenta. From the eighteenth day onwards the maximum shortening of the uterine muscle cells caused by the pituitary extract was just sufficient to produce destruction of the attachment of the placenta; and in the last three or four days of pregnancy (the twenty-ninth to the thirty-second) delivery of the foetus

was easily brought about. The dose required, however, was progressively greater in passing backwards from the thirty-second to the earlier days.

Knaus has done well to call attention to what is evidently an essential factor in parturition—namely the required development of the uterine muscle which at the end of pregnancy is very great in all placental mammals. It is equally clear, however, that the muscular hypertrophy is not the sole factor. Knaus proceeds to quote the experiments of Saurbruck and Heyde who united together two rats by the method of parabiosis. Five experiments were carried out, each rat in a pair being at a different stage of pregnancy. In each of two experiments one of the partners produced young without affecting the other partner whose young were not born until a fortnight later. This result is attributed by Knaus to the uterine muscles of one of the partners in each experiment not being sufficiently developed. In the other three experiments in which the stages of pregnancy of the partners were closer, parturition took place simultaneously in each of the two rats, one partner having a litter of normal young while the other aborted a litter of undersized foetuses. These experiments afford a confirmation of the view adopted here that the working of an endocrine mechanism is a normal factor in parturition. It may be pointed out, further, that Knaus's theory affords no explanation of the remarkable phenomena attending the closing phases of pseudo-pregnancy in the various species referred to above.

Other factors in parturition.

It would seem possible that in any given species the degree of development of the young and the distension of the uterus may be a factor in parturition. On the other hand, there is often much variation in the size and weight of the newly born, and twins and triplets are not usually produced after a shorter pregnancy than single offspring.

It has long been known that the nervous system plays an important part in normal labour, and there is a presiding centre located in the lumbar part of the spinal cord. Normally the course of parturition depends upon the integrity of this centre and of the nerves supplying the muscles of the uterus and those of the abdominal wall. Nevertheless, parturition has been shown to occur in the absence of the nervous mechanism as in Simpson's experiment on a sow and those of Goltz on a bitch, in both of which the dorso-lumbar portion of the cord was exsected. In these cases, however, labour did not proceed normally owing to the absence of co-ordination of the muscular movements. Parturition has also been known to take place in women suffering from paraplegia, as in Brachet's case and Routh's case. The integrity of the nervous system must therefore be regarded as one of the normal conditions for the occurrence of labour, but like other of the factors involved in the process it is not indispensable.

There is evidence that parturition may sometimes occur also in the absence of the ovaries, for cases have been recorded in which ovariotomy was performed in the later stages of pregnancy and yet labour took place at the normal time. Hammond however states that in the rabbit, removal of the ovaries is invariably followed by abortion even in the last days of pregnancy, and Drummond-

Robinson and Asdell have obtained the same result in the goat by excising the corpora lutea. In those cases in which abortion does not happen it is possible that some compensating mechanism may have come into existence, for it is known that there is a functional inter-relation between the various endocrine organs of the body. For instance the pituitary undergoes hypertrophy after ovariotomy, but this hypertrophy is believed to be confined to the anterior lobe; it may be pointed out, however, that there is some evidence of a functional connexion between the anterior and posterior lobes.

General conclusions.

The duration of gestation depends upon a number of conditions which, though generally constant within a particular species, vary widely in different species and even among closely related forms. Individual variation also is commoner than is often supposed; thus, prolonged gestation as a result of suckling is frequent among various species of rodents. Of the different factors which contribute to the immediate cause of parturition the phase of the ovarian rhythm is one of the most important. It has been shown that the process usually recurs when the corpus luteum has entered into a stage of regression, and therefore may be supposed no longer to dominate the ovarian metabolism. The phenomena attending and following pseudo-pregnancy afford further evidence that this is the case. In view of the specific influence of pituitary extract upon the muscles of the uterus it is probable that this gland plays a part in bringing about parturition, and experimental evidence has shown that there is a functional correlation between the ovary and the pituitary, the secretion of the former acting upon the latter in the absence of the corpus luteum. It has been shown further that the degree of development of the uterine muscles is a factor in parturition and that these undergo a marked growth more especially in the final phase of pregnancy. The development of the foetus *in utero* may also be a factor in the stimulation of the uterine muscles. The integrity of a centre in the lumbar region of the spinal cord and of the nerve connexions of the uterus and abdominal wall is essential for the normal expulsion of the foetus, but nevertheless parturition may occur in the absence of this integrity. Similarly, parturition may abnormally occur in the absence of the complete endocrine mechanism which is usually an important factor in the process, and it is possible that compensatory mechanisms may come into being when the normal ones have been experimentally interfered with.

[Postscript, January 1927]

Since writing the above I have seen Blau and Hancher's recently published paper on the uterine contracting power of spinal fluid after the administration of certain tissue extracts. The experiments were on substantially the same lines as those of Dixon and Marshall. They show the presence of an oxytocic substance in spinal fluid after injecting ovarian interstitial extract but testis, liver and spleen also gave positive results. Oral administration of dried ovarian powder was likewise positive. Extract of corpus luteum gave uncertain results. Water soluble extracts of ovaries etc. gave more markedly positive results than fat soluble ones.

BIBLIOGRAPHY.

ABEL (1924). *Johns Hopkins Hospital Bull.* **35.**
ANCEL and BOUIN (1910). *Journ. Physiol. et Path.* **12.**
—— (1912). *C. R. de l'Acad. des Sci.* **154.**
BLAIR BELL (1919). *The Pituitary.* (London.)
BLAU and HANCHER (1926). *Amer. Journ. Physiol.* **77.**
BRACHET (1837). *Recherches.* 2nd ed. (Paris.)
BURN and DALE (1922). *Reports on Biological Standards.* (Med. Res. Council, London.)
COW (1915). *Journ. Physiol.* **49.**
CUSHING (1926). *Studies in Intracranial Physiology and Surgery.* Oxford and London.
CUSHING and GOETSCH (1910). *Amer. Journ. Physiol.* **27.**
DIXON (1923). *Journ. Physiol.* **57.**
DIXON and MARSHALL (1924). *Journ. Physiol.* **59.**
DONCASTER (1913). *Proc. Camb. Phil. Soc.* **17.**
DRUMMOND-ROBINSON and ASDELL (1926). *Journ. Physiol.* **61.**
FOSTER (1890). *Textbook of Physiol.* Pt 4. 5th ed. (London.)
GOLTZ (1874, 1896). *Pflüger's Arch.* **9, 63.**
HAMMOND (1925). *Reproduction in the Rabbit.* Edinburgh.
HAMMOND and MARSHALL (1914). *Proc. Roy. Soc.* B, **87.**
HARTMAN (1923). *Amer. Journ. Anat.* **32.**
HERRING (1908). *Quart. Journ. Exp. Physiol.* **1.**
HILL and O'DONOGHUE (1913). *Quart. Journ. Micr. Sci.* **59.**
ITAGAKI (1917). *Quart. Journ. Exp. Physiol.* **11.**
KNAUS (1926). *Journ. Physiol.* **61.**
LESCHKE (1919). *Biochem. Zeit.* **96.**
MARSHALL and HALNAN (1917). *Proc. Roy. Soc.* B, **89.**
MAYER (1924). *Berlin. Klin. Woch.* **3.**
MIURA (1925). *Pflüger's Arch.* **207.**
OEHME (1918). *Deutsch. Arch. f. klin. Med.* **127.**
PARKES and BELLERBY (1926). *Journ. Physiol.* **62.**
ROUTH (1898). *Trans. Obstet. Soc.* **39.**
SAURBRUCK and HEYDE (1910). *Münch. med. Woch.*
SIMPSON (1871). *Selected Obstetric Works.* (Edinburgh.)
TRENDELENBURG (1925). *Berlin. Klin. Woch.* **3.**
WILLIAMS (1923). *Obstetrics.* 5th ed. (New York and London.)

THE MECHANICS OF VERTEBRATE DEVELOPMENT

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(With 11 Text-figures.)

CONTENTS.

1. INTRODUCTION.

IT is proposed in this article to review the work which has been done and the results which have been achieved in the experimental study of the early development of vertebrate animals by the already large number of investigators in all quarters of the globe.

Roux, to whom the science of developmental mechanics owes so much, distinguished two main periods in development: an earlier one in which the organs appear without function, and a later one in which function conditions the further development of the organs. These pages will concern only the earlier period. The experimental results obtained in connection with functional differentiation and adaptation are therefore purposely excluded from this review, as also are those dealing with hormone action, the action of abnormal environments and with regeneration.

The present scope therefore is limited to an analysis of the processes whereby in normal development the various rudiments of the vertebrate embryo are

determined and make their first appearance. The strides which have been made in this study are due to the invention or perfection of new methods. Experiments in this field are no longer "mass-performances" with statistical results, but are more often microsurgical operations on individual embryos. The fine needle as used by Roux (1895 *b*) and others, enables a definite region to be destroyed. A child's hair can be used as a ligature to separate two blastomeres, as Herlitzka (1897) and Spemann (1921 *a*) have shown. To the latter is also due the micropipette with which definite and minute regions of an embryo can be removed and transplanted to another embryo with great precision. Glass needles, hair loops, very fine knives, Peterfi's (1924) microcauteriser and Chambers' (1922) microdissection apparatus complete this microsurgical equipment.

Other methods have for their object not the removal but the identification of certain regions so as to be able to follow their displacements during development. For this purpose, the methods of *intra vitam* staining elaborated by Goodale (1911), Detwiler (1917) and Vogt (1925) are pre-eminent.

Yet other methods aim at studying the behaviour of definite regions when completely isolated from the organism. To this end they may be explanted as tissue cultures *in vitro* in suitable media, or in the case of birds, grafted on to the chorio-allantoic membrane of another developing egg, by methods due to Peebles (1898), Murphy and Rous (1912), Murphy (1916), Danchakoff (1916) and Minoura (1921). *In vitro* methods for such morphogenetic rather than histological study have been elaborated by Braus (1911), Harrison (1912), and by Strangeways and Fell (1926).

The material worked upon necessarily bears a relation to the methods mentioned, and consists chiefly of frogs, toads, newts, chicks and a few fish. Nevertheless, so many "complex components" of development have been discovered that it is possible to obtain a general idea of the causal connections between many of the important developmental processes.

For previous general or partial surveys of this field the reader may be referred to the following works: Jenkinson (1913, 1917), Dürken (1919), Petersen (1923, 1924), Brachet (1917), Spemann (1919, 1924), Mangold (1925), Huxley (1924), Duesberg (1926), de Beer (1926), Przibram (1926).

It goes without saying that, in what follows, the comparative embryology of vertebrates is taken for granted, except in so far as certain experiments have shed more light on various processes.

2. PROLEGOMENA.

It is necessary in the first place to have a clear idea of the structure and potencies of the egg, that of Amphibia being chosen as an example. When laid, the frog's egg although spherical possesses an axis, since yolk is accumulated at one (the vegetative) pole, and protoplasm and pigment at the other (the animal) pole. Yolk having a higher specific gravity than protoplasm, the axis of the egg is normally vertical with the animal pole on top. Gravity is however not responsible for the formation of this axis, as the following experiment of Roux (1895 *a*) shows.

The action of gravity can be eliminated by putting the egg in a clinostat which

revolves slowly about a horizontal axis. The eggs are constantly tumbling over one another and therefore do not present the same pole to the centre of the earth for any length of time. Nevertheless normal larvae develop. The axis must have been determined previously, before the egg was laid. Gravity cannot be invoked at these early stages either, for in the ovary there is no definite orientation. Bellamy (1919, 1921) has been able to show that the capillaries of the follicle probably determine the axis of the egg since at early stages the animal hemisphere is that to which arterial blood is supplied, while the venous blood is removed from the region of the vegetative hemisphere. This is not necessarily true for later stages however. The prime differentiation is therefore impressed from without, for the animal pole will become the head of the embryo.

The bilateral symmetry of the egg is brought about for the most part by the entrance of the sperm (Roux, 1903; Jenkinson, 1909) in normally fertilised eggs. In frogs, the grey crescent is formed at the meridian antipodal to that where the sperm enters, by retreat of pigment to the interior. That the two events are connected follows from Herlant's (1911) observation that in frogs' eggs into which two sperms have entered, the grey crescent appears at the antipode to the meridian half-way between the points of entry of the two sperms. At the same time Jenkinson (1909) showed that the plane of symmetry of the egg might in certain cases be determined by incident light and by gravity. Both these factors are external to the egg. Since the dorsal lip of the blastopore begins to form in the centre of the grey crescent, it may be said that the sperm fixes the "Greenwich" meridian of the egg (again from without) and determines the future dorsal, ventral and right and left sides. No grey crescent is found in the newt's egg, but a region with characteristic pigmentation appears in the axolotl after fertilisation (less distinctly in *Triton*), in which the dorsal lip of the blastopore later arises. This was proved by *intra vitam* staining marks by Vogt and Goerttler (quoted by Bautzmann, 1926).

The plane of the first furrow is at right angles to the first spindle and this spindle is at right angles to the later or "copulation" path of the sperm (Roux). If the "penetration" and "copulation" paths of the sperm are in the same straight line, then the plane of the first furrow will coincide with the plane of bilateral symmetry. If on the other hand the copulation path is deflected, the plane of the first furrow may make any angle with the symmetry plane. Jenkinson (1909) was able to study these relations in horizontal sections of the two-cell stage, the path of the sperm being indicated by a trail of pigment. Roux's method was to cause the sperm to enter the egg in a definite meridian by means of experimental devices such as a fine pipette or a thread leading the sperm to the egg.

In newts, by placing a ligature round the egg in the plane of the first furrow and observing the relation which it makes with the dorsal lip of the blastopore when it appears, Spemann found that the first furrow tends either to coincide with or to be at right angles to the plane of bilateral symmetry. By means of *intra vitam* stains used as reference marks, Vogt (1923) concludes that there is more irregularity in the relation of the planes than Spemann supposes. The importance of these matters will be apparent in what follows.

With regard to the normal process of gastrulation, experiments have settled two important points; first the relative contributions of ingrowth and epiboly to gastrulation in Amphibia, and the question of concrescence as a method of closure of the blastopore.

In *Amphioxus*, the egg of which contains little yolk, gastrulation takes place by invagination as a result of which the diameter of the blastopore is at first nearly that of the whole blastocoel, and it subsequently becomes reduced and closed by growth of the blastopore rim. In Amphibia, epiboly takes place certainly as a means whereby the dark cells of the animal hemisphere come to cover the lighter coloured
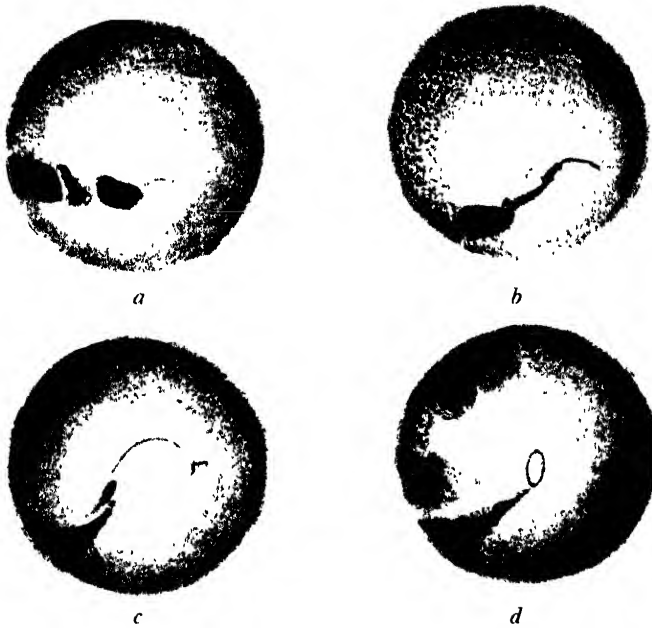
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Fig 1. Embryo of *Pleurodeles* during gastrulation with *intra vitam* reference marks. The marks get carried to the rim of the blastopore, turn in over its edge, and are seen in *d* by transparency through the ectoderm, moving away from the blastopore on the inside. (From Goerttler.)

cells of the vegetative pole, but owing to the large amount of yolk present, typical invagination as in *Amphioxus* cannot occur, and some have doubted whether it occurs at all. By placing reference marks of *intra vitam* stains on the surface of blastulae of *Triton* and *Pleurodeles*, Goerttler (1925) observed that they disappeared over the edge of the lip of the blastopore and could be followed by transparency moving away from the blastopore on the inside. There is no doubt therefore that ingrowth does occur, a matter of great importance in subsequent differentiation. Vogt (1922 *c*) observed that the material at the rim is constantly changing by rolling in, and is supplied by new cells which move towards the blastopore on the outside. The maximum ingrowth takes place in the mid-dorsal line, and *intra vitam* reference marks show that on the surface, in the region between the animal pole and the dorsal lip of the blastopore, pronounced stretching takes place in the meridional plane.

To a lesser extent the stretching and rolling in takes place all round the blastopore rim. The nearer a mark is to a rim of the blastopore at the start, the further forward does it get pushed inside.

By means of transplantation experiments to be detailed later, Mangold (1924) showed that the different regions of the blastula have certain tendencies towards "mass movements," the results of which are the process of gastrulation. So the cells of the animal hemisphere, especially on the dorsal side, tend to grow and increase their surface; those in the region of the future blastopore rim tend to grow in, while the vegetative pole regions are more passive. It is worth while noticing that as the lips of the blastopore, the ring of overgrowth, are below the equator, the process of epiboly entails a contraction of this ring corresponding to the decreasing diameters of latitudes approaching the vegetative pole. Vogt (1922 *a, b*) removed a portion of the roof (animal pole) of the blastula of *Triton*. The gap closed by approximation of the sides of the wound. But by this process the future ring of overgrowth was drawn up above the equator. When gastrulation started this rim attempted to grow over and at the same time to decrease its diameter. The result
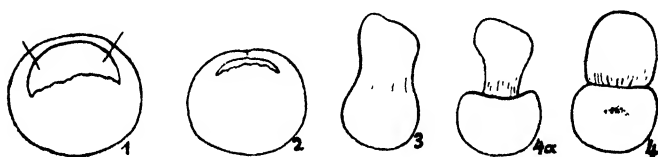
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Fig. 2. Diagrams illustrating experiments on *Triton* embryos. 1. A portion of the roof of the blastula is removed; 2. The wound closes over by approximation of the sides. 3. A constriction develops, due to the decreasing diameter of the presumptive blastopore-rim, which is drawn up above the equator by the closure of the wound. 4a. The constriction becomes accentuated 4. A separate invagination takes place in the region of the yolk cells. (From Vogt.)

was a constriction causing the embryo to resemble an hour-glass. At the same time, beneath this rim which represents the margin of overgrowth, and separate from it, a simple pit is formed by invagination in the yolk cells. By the experiment, therefore, these two processes were more or less dissociated. On the other hand, by treating frogs' eggs with LiCl, $CaCl_3$, sugar or sodium acetate, Giersberg (1924) obtained gastrulae in which invagination had occurred, but no epiboly, for the blastopore remained enormous.

After completion of gastrulation, the continuance of the movements which brought it about play an important part in the formation of the tail bud. (Vogt 1926.) As regards the supposed closure of the blastopore by the concrescence in the middle line of laterally situated material, Goodale (1911), by means of *intra vitam* staining, found that the movement of material in *Spelerpes* and *Amblystoma* took place along meridional lines, *i.e.* parallel to the mid-dorsal line, and not towards it. The same thing has been shown by Vogt (1922 *c*) and Goerttler (1925) for *Triton* and *Pleurodeles*, and by Smith[1] (1914) for *Cryptobranchus*. In selachian embryos, Kastchenko (1888) found that injury to the edge of the blastoderm was

[1] Smith's interpretation of his results is slightly different.

not carried to the middle line, but remained on one side. Lewis (1912) working on *Fundulus* found the same thing; indeed here a side of the germ ring can be removed without interfering with the blastopore or the embryo. If concrescence occurred, injury to the dorsal lip of the blastopore in the middle line should not prevent the more lateral tissue from growing towards the middle line further back; Kopsch (1896) found in the trout that it did prevent it. Lastly, a very elegant experimental disproof of concrescence has been given by Vogt (1923). If concrescence does not occur, then those cases of spina bifida, when the blastopore, *e.g.* of Amphibia, does not close properly must be due, not to the failure of the lateral

b′   *b*
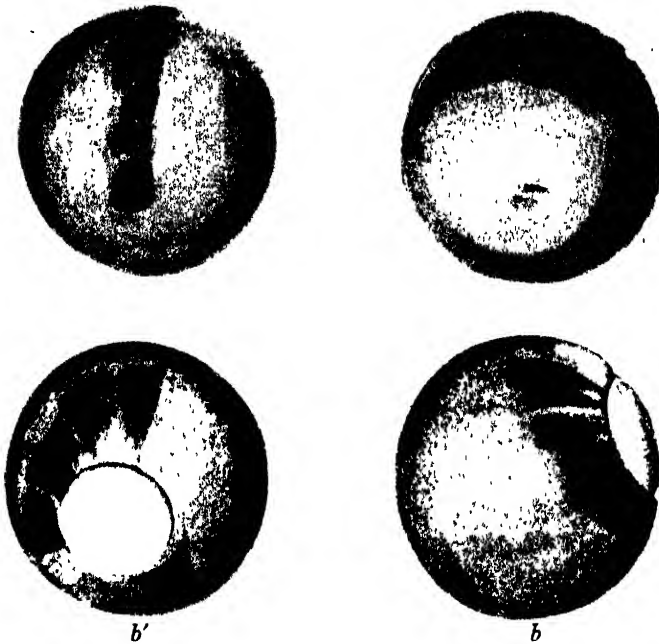
(Reproduced with permission from *Zeitschrift für wissenschaftliche Biologie*, Bd **106**)

Fig. 3. Embryo of *Pleurodeles* with *intra vitam* reference marks. *a* and *b*, lateral views; *a′* and *b′*, views from the vegetative pole; *a*, early stage; *b*, late stage of gastrulation. The marks move along meridional lines to the blastopore rim. (From Goerttler.)

tissue to reach the middle line, but to the displacement to the sides of tissue normally in the middle line. To prove this, an *intra vitam* reference mark was placed in the centre of the dorsal lip of the blastopore at the outset of gastrulation in a newt's egg. Spina bifida or "Asyntaxia" was then induced by artificial means (certain solutions are capable of effecting this) and it was found that the reference mark had been split into two and displaced on each side away from the middle line.

As the diameter of the blastopore decreases during gastrulation, so points on its rim get nearer together. This is not concrescence but convergence.

The *intra vitam* staining method can be used in the chick, as by Wetzel (1925), who showed that a mark at the posterior edge of the blastoderm stretches forward in the formation of the primitive streak.

3. DETERMINATION BEFORE AND DURING CLEAVAGE.

It is necessary to consider the facts relating to the potency and restrictions of potency, or determination, of the egg and the blastomeres into which it divides during cleavage.

Roux (1895 *b*) killed one blastomere of the 2-cell stage of the frog, by means of a hot needle. The result of this classical experiment was that the remaining blastomere developed into a half-embryo; usually a hemiembryo lateralis, or in some cases a hemiembryo anterior. It looked as if the blastomere gave rise only to those structures which it would have produced in normal development, and that it was already determined as regards its fate. On this experiment and on Weismann's speculations, rests the Roux-Weismann theory of mosaic development, which regarded the division of nuclei as responsible for the differential determination of the different blastomeres. The fact that some of Roux' hemiembryos later on completed themselves by postgeneration does not affect the present argument.

Hertwig (1893) challenged Roux' conclusions and showed that differentiation could not rest on unequal nuclear division between the blastomeres. By compressing frogs' eggs in different ways, he completely altered the normal distribution of the nuclei and nevertheless obtained perfect embryos. From his experiments of injuring one blastomere he concluded that Roux' half-embryos were produced owing to the dead blastomere remaining in contact with the living one, and prophesied (successfully) that if the dead one were removed, the other would reveal itself totipotent, and produce a whole embryo. The variability of the results of killing one blastomere also weighed against the probability of a determined mosaic development.

The analysis was carried a step further by Schultze (1894), who reversed frogs' eggs at the 2-cell stage so that their light-coloured vegetative poles were uppermost. The result was double monsters, *i.e.* each blastomere produced much more than half an embryo, which was its normal prospective fate. Wetzel (1895) confirmed these results and showed that after reversal a reorganisation of each blastomere takes place, the yolk sinking down along the cell-wall dividing them and the protoplasm rising, as Born (1885) had shown to be the case with the egg. These double monsters have a common blastopore. The reorganisation and rearrangement of the contents of the blastomeres are therefore responsible for restoring to them the original potency of the egg.

Morgan (1895) adapted these results to Roux' experiment and showed that when one blastomere was killed, if the other were left animal pole uppermost it developed into a half-embryo, if it were reversed so as to lie vegetative pole up it developed into a whole embryo of small size.

Hertwig's prophecy was verified by McClendon (1910), who removed one blastomere of the 2-cell stage of the frog *Chorophilus triseriatus* by sucking it out with a fine pipette. The remaining blastomere became a small whole embryo.

There is no doubt therefore that in the frog the prospective potency of the blastomeres is greater than their prospective fate, and the potency of the blastomeres of the 2-cell stage is, except for one important reservation, equal to that of the

egg. In order to develop, a blastomere must contain a portion of the grey crescent, or future blastopore lip. Brachet (1917) showed that Schultze's double monsters are only obtained if the planes of the first furrow and of bilateral symmetry nearly coincide; the result of which is that each blastomere has some of the grey crescent. Again, it has already been mentioned that such monsters have a common blastopore.

There is therefore an important determination already present in the frog's egg; the grey crescent. In addition there is evidence of a reversible determination of certain regions. Brachet (1905) observed that when one blastomere was killed and the other allowed to develop without disturbing it, the resulting half-embryo was orientated with regard to the grey crescent. It was either a lateral half, an oblique half or an anterior half, according to the angle between the planes of the first furrow and of symmetry. Brachet (1906) further discovered that by making a small injury in an egg up to 45 minutes after fertilisation no effect was produced; between 45 and 60 minutes after fertilisation, it resulted in an asymmetry on the injured side, which means that a quantitative deficiency on that side had not been made good. Injury from 60 to 90 minutes after fertilisation resulted in qualitative deficiency of some region. Morgan (1905) removed material from the animal pole at the 2-cell stage and observed that the resulting embryos were deficient in the dorsal region. By acting with ultra-violet light on frogs' eggs in the 1- or 2-cell stage, Baldwin (1915, 1919) produced quantitative defects (asymmetry) without qualitative or histological deficiencies.

Dürken's (1925 b and 1926) experiments are of the greatest interest in this connection. In order to answer the question whether tissues which have not been subjected to the action of the blastopore lip can differentiate, he took pieces from the region of the animal pole of blastulae and gastrulae of *Rana fusca*, and grafted them into the cavity of the orbit of older embryos. Pieces from blastulae differentiated (of course abnormally yet unmistakably) into notochord, cartilage and ganglion cells which formed ganglion-like masses. In another case he obtained differentiation of a portion of the brain and auditory vesicles. A piece from a young gastrula gave a limb-like structure containing cartilage, bone, muscle, nerve cells, blood vessels, glands and connective tissues. The tissues of the host took no part in these structures.

These experiments show that in addition to the irreversible grey crescent, frogs' eggs have from an early stage certain determinations which are reversible at first (*teste* whole embryos from one blastomere) and which are *cytoplasmic*, not nuclear.

In the newt's egg, Herlitzka (1897) showed that the blastomeres of the 2-cell stage could be separated by ligaturing between them with a fine hair, and that each could produce a perfect embryo. These results have been confirmed and amplified by Spemann (1901 a, 1902, 1903), who found that incomplete constriction produced duplicitas anterior, and that complete constriction could be followed by the formation of a pair of normal small embryos, even as late as the early gastrula stage, provided always that the plane of constriction (a) was meridional, and (b) coincided with that of symmetry. In other words each blastomere or half-gastrula must contain a portion of the future dorsal lip of the blastopore. If the constriction is

made transversely, one product will contain this blastopore region and will develop: the other will lack it and will never develop beyond forming the three germ layers. After obtaining separate blastomeres of the 4-cell stage, Ruud (1925) observed that two of these developed into normal miniature larvae whereas the other two (which lacked the blastopore-lip-region) did not develop at all.

The converse experiment to obtaining wholes from portions is to obtain a single whole from two conjoined embryos. This remarkable feat was performed by Mangold (1920) by placing two 2-cell stage embryos of *Triton* crosswise over one another, after freeing them from their membranes. If the two blastopore-dorsal-lip-regions did not come to lie adjacent to one another, the result was a double monster. If on the other hand they did come together, a single normal giant embryo was formed.

These results show that in the newt's egg the region of the future dorsal lip of the blastopore is the only determined region, before and during cleavage. No other regions are determined at these stages. Except for the dorsal-lip-region, newt's eggs and blastomeres regulate. Still more so do the blastomeres of the fish *Fundulus heteroclitus*, the potencies of which were tested by Lewis (1912). Injury with a needle to the undivided egg within 2 hours of fertilisation gave rise to normal small embryos. Destruction of one blastomere of the 2-cell stage does not prevent the other from forming a normal small embryo. At the 4-cell stage removal of any one blastomere results in the remaining three forming a normal embryo; and if two blastomeres are removed, the remaining two, provided that they are not a diagonal couple, will do likewise. Even at the blastoderm stage injuries fail to induce qualitative abnormality in the resulting embryos.

The result of these enquiries is to show that in the amphibian egg, the region destined to form the dorsal lip of the blastopore is a determined region of extreme importance, and that at the early stage here considered other determinations are either non-existent (newts and *Fundulus*) or labile (frogs) up to a point. At all events, such determinations are cytoplasmic and not nuclear. This fact, which has now been established in most groups of the animal kingdom, is of the greatest importance. No demonstration of it could be more elegant than that of Spemann (1914). By placing a ligature round an egg of *Triton* before cleavage begins, and dividing the egg incompletely into two halves, the nucleus can be confined to one side of the constriction. After the nucleus has divided a certain number of times on that side the constriction can be released to allow one product of division to cross to the other side. If the constriction was in the symmetry plane, each of the lateral halves can develop, whether it contains one sixteenth or fifteen sixteenths of the original cleavage nucleus (Spemann, 1924). If, however, one sixteenth of the original cleavage nucleus is allowed to cross the bridge from the ventral to the dorsal half, the latter will not develop. This means that after the nucleus has divided three times in the ventral half, its products are incapable of functioning normally in the dorsal half. That this incapacity is due to a cytoplasmic effect on the nucleus is proved by the fact that, in lateral halves, a one-sixteenth nucleus is perfectly capable of functioning so as to produce a normal embryo. This result is obtained

by severing all connection between the blastomeres after the nucleus has passed across. If they are allowed to remain in contact a double monster is produced, the components of which differ in "age."

Lastly, if a (lateral or dorsal) half, from which the cleavage nucleus and its products have been completely excluded, happens to contain an accessory sperm, it will develop into a haploid but otherwise normal embryo.

Haploid embryos can also be obtained by exposing the eggs or the sperms to radio-active substances before fertilisation. G. Hertwig (1913) obtained embryos from eggs of *Rana esculenta* and *Bufo vulgaris* which had been fertilised with irradiated sperm of *Rana fusca*. P. Hertwig (1913) showed that after having been radiated the sperm nucleus plays no part in such development, which is therefore a kind of parthenogenesis. The failure of species crosses such as these to produce normal embryos when normal sperm is used is therefore due to the incompatibility of the nuclear materials of egg and sperm. O. Hertwig (1913) obtained embryos from *Triton* eggs fertilised with irradiated sperm of *Triton* and of *Salamandra*. P. Hertwig (1916, 1924) subjected the eggs of *Triton* to radiation and fertilised them with normal sperm and obtained embryos.

The haploid nature of these embryos was proved by counting their chromosomes and measuring their nuclei. Haploid tissue can therefore always be recognised, and G. Hertwig (1925) has made use of this fact to recognise and identify grafts and the structures to which they give rise apart from the tissues of the host.

It is worth mentioning that Lewy (1913) was able to rear embryos of *Rana esculenta* and *temporaria* (which had been activated by Bataillon's (1904) pricking method) past the stage of metamorphosis into little frogs.

4. DETERMINATION DURING GASTRULATION.

Spemann (1903) observed that when embryos of *Triton* were divided or constricted in the sagittal plane in the blastula and up to the early gastrula stages, normal twin embryos and duplicitas anterior depending on the degree of constriction could be obtained. At later stages however no such regulation of the parts took place. The time of determination must therefore lie somewhere between the beginning and end of gastrulation. Additional evidence is obtained from cases of twinning in other animals. Stockard (1921) exposed late cleavage stages of *Fundulus* to low temperatures (5° C.), and in other experiments deprived them of oxygen. The result was twins and double monsters. After gastrulation is completed however, the development can be stopped or inhibited with comparative impunity. Newman (1923) as a result of study of twinning in the armadillo concludes that the gastrula is the critical stage. Lebedinsky (1923) correlates the frequency of twinning in Teleosts, Reptiles, Birds and Mammals with their blastoderms and flat gastrulae; as contrasted with the Cyclostomes, "Ganoids" and Amphibia in which the gastrula is spherical and twinning is infrequent. The point is that no twinning or regulation takes place after gastrulation has been completed.

Spemann tested the determination of different regions of the newt at different stages by means of transplantations (1918). In the following descriptions the word

"presumptive" is used to mean the prospective fate of any given piece of tissue in normal development. At the beginning of gastrulation a piece of presumptive epidermis (from the flank or ventral region) was exchanged for a piece of presumptive nerve tube. The pieces differentiated according to their new surroundings and regardless of their origin. This proves that at this stage they are not irrevocably determined. It is interesting to notice that a ventral piece can become nerve tube, although a ventral half-gastrula deprived of the dorsal lip region will not differentiate. What the ventral half lacks therefore is not potency but a factor, which will be considered in the next section.

The transplantation experiment can be made still more striking by using different species or genera as "donors" and "hosts." The tissues of *Triton cristatus* are light and free from pigment, those of *Triton taeniatus* are dark; they can therefore readily be distinguished (Spemann, 1921). A piece of presumptive *cristatus* epidermis in *taeniatus* anterior nerve-tube region will differentiate into an eye cup. A similar piece in the otic region of a *taeniatus* embryo will develop into a proper ear. *Bombinator* presumptive epidermis grafted into the anterior nerve-tube region of *Rana esculenta* will give rise to an eye cup, the lens corresponding to it being formed of *esculenta* tissue. A very interesting case is that in which a piece of *taeniatus* presumptive nerve-tube region is grafted on to the flank of a *cristatus* embryo. The graft differentiates into gills according to its new position, but it preserves its *taeniatus* character in that these gills are larger and better developed than the normal *cristatus* gills on the other side of the embryo. This difference at the same stage is observable between the two species. In other cases (Spemann, 1918) in which a graft of presumptive epidermis from a younger donor is grafted into the anterior nerve-tube region of an older host, the graft differentiates into an eye cup. This eye cup is smaller than the normal other one of the host, but is similar in size to the remaining one in the donor. While therefore there is no determination at this early gastrula stage, the tissues nevertheless preserve some of their original peculiarities.

An exchange between presumptive epidermis and nerve-tube pieces in an older gastrula of *Triton*, 21 hours before the neural folds appear (room temperature), leads as before to differentiation according to the new position of the pieces, only this time less completely. If the exchange is performed on a still older gastrula, when the blastopore is a slit and 9 hours before the neural folds appear, the pieces differentiate according to their place of origin, and thereby show that they have been irrevocably determined at this stage. Determination in respect of the nerve cord therefore sets in in *Triton* towards the end of gastrulation.

Confirmation of this is found in the power of regulation at different stages of constricted dorsal half-gastrulae of *Triton*, studied by Ruud and Spemann (1923). These dorsal halves contain the dorsal lip of the blastopore and consequently are capable of development. Should they be constricted off at the early gastrula stage, the resulting embryo is perfect and has a properly proportioned nerve tube for its size, although it contains the material which in normal development goes to make a nerve tube double this size. At this stage therefore the dorsal half-gastrula is

still capable of regulation, and the nerve tube is not yet determined. If the dorsal half is constricted off at a later stage when the blastopore is small and oval, yet still open, regulation no longer takes place and the resulting embryo develops with neural folds which are relatively much too large for it. It is worth noticing that whereas the power of regulation ceases at this stage with small, oval yet open blastopore, transplantation experiments prove that the definite determination of the neural tube does not take place until a slightly later stage, when the blastopore is closed and slit-like. This slight difference in time is to be expected and means that the processes of regulation themselves take a certain time. If they are initiated too late, the structures will be determined before the processes have power to act. If the time discrepancy were the other way, it would be inexplicable.

In *Fundulus*, Lewis (1912) found that determination had taken place at the comparable embryonic shield stage. An injury to the posterior end of the shield prevented the formation of the tail and hinder region of the body; an injury at the anterior end prevented the formation of the head.

In newts and *Fundulus*, therefore, the parts of the embryo cease to be equipotential, and the neural tube is determined at a late gastrula stage.

Before leaving this subject, attention must be paid to some other interchanges of regions before determination sets in. By means of *intra vitam* reference marks, Goerttler (1925, 1926) has shown which regions of the blastula (when the dorsal lip of the blastopore is just appearing) are to be regarded as presumptive ectoderm, presumptive notochord, presumptive endoderm and presumptive mesoderm. Mangold (1924) has carried out extensive potency tests on these regions at the blastula and early gastrula stages. He found that a piece of presumptive ectoderm could become part of notochord, somite, pronephros, splanchnopleur, somatopleur and gut wall, according to the position it reached during gastrulation, which again depended on the time and place where it was grafted. The first four possibilities enumerated above followed the implantation in the blastopore lip, the last resulted from placing in the blastocoel. Presumptive mesoderm can be made to form ectoderm. Presumptive endoderm does not do well in an ectodermic region owing to its passivity and tendency to become overgrown. For although the various regions at this stage are not restricted in their potency, yet they are distinguished by tendencies which lead to the normal process of gastrulation: growth and extension of surface of the animal pole region, growth and inflexion of the blastopore rim and passivity of the vegetative pole region. Transplanted portions "behave" as they would have done if left alone, but they differentiate according to their new positions. It is interesting to compare this phenomenon with those already mentioned as regards retention of certain specific and age characteristics (size of eye and gill in *Triton*) and with the cases among Invertebrates of isolated blastomeres cleaving as parts and developing as wholes. After the yolk-plug stage is reached, these mass-movement tendencies disappear. For the rest, these implants tend to adapt their cell size and division speed to their surroundings, but not always; sometimes the grafts grow much more rapidly than their surroundings. It may be added that the recognition of the implants in the various situations was guaranteed by the heteroplastic method (*Triton taeniatus* and *cristatus*).

5. THE ORGANISER.

During the experiments just described dealing with the determination of the neural tube at the gastrula stage in *Triton*, Spemann (1916) observed that in some cases while the presumptive neural tube material in an anterior region might still be indifferent, that at the hinder end near the dorsal lip of the blastopore was already determined. This looked as if a "flow of determination" started from a definite place, the dorsal lip of the blastopore, and spread thence over the embryo. This suspicion is further supported by the fact that if in the early gastrula stage of *Triton* the animal hemisphere be cut off, rotated through 90° or 180° about the egg axis and stuck on again, the neural folds arise in a line in front of the dorsal lip of the blastopore (situated in the ventral hemisphere) from material which would normally never have formed them. At the same time, from the original presumptive neural fold material which has been rotated away, neural folds do not form (Spemann, 1906 *b*, 1918). Further, if two gastrulae are divided sagittally and the two right halves stuck together on the one hand and the two left on the other, each composite embryo has two half-blastopores, one on each side. Each completes itself into a whole blastopore with the result that double monsters are formed from each embryo. The determination and differentiation of the embryo therefore appear to be dependent on the dorsal lip of the blastopore. This was proved beyond doubt by Spemann and Mangold (1924), who transplanted a piece of the dorsal lip of the blastopore of one embryo into the flank of another at the gastrula stage. Here the implant induced the formation of a second embryo with nerve tube, auditory vesicles, notochord, somites and pronephros. Some of the structures of this secondary embryo were formed from the host's tissue, and some from the graft. The notochord was always formed from the graft, the nerve tube mostly from the host cells, somites could be formed from either alone or both together. In some of Bautzmann's (1926) experiments the secondary embryo was as perfectly formed as the primary. From this power which the dorsal lip of the blastopore possesses of organising the main structures of an embryo, it has been given the name of *Organiser*.

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Fig. 4. Left side view of an embryo of *Triton taeniatus* into which an organiser has been grafted. The figure shows the dorsal side of a well-formed secondary embryo. Note that the auditory vesicles of the primary and secondary embryo are at the same level. (From Spemann.)

Geinitz (1925 *a*) carried this astounding analysis further when he showed that the organiser could work not only in grafts between different species and genera, but also between subclasses. Organisers of *Pleurodeles waltli* and of *Amblystoma mexicanum* induced secondary embryos in *Triton taeniatus*. When an organiser from *Bombinator pachypus* was grafted into *Triton taeniatus*, a neural tube, auditory vesicles and somites were induced from the tissues of the host, and at the same time the graft itself differentiated into another nerve tube, a notochord and mesenchyme. The reason for this duplicity of the nerve cord is that there is too much incom-

patibility between the tissues of *Bombinator* and *Triton* to contribute together to form one organ. While in the organiser grafts between *Triton cristatus* and *taeniatus* both took part in the formation of a single nerve tube, in the case in question each tissue differentiated by itself since "chimaeras" cannot be formed. This case is further interesting as showing that the action of the organiser is neither by self-differentiation nor by induction alone.

It should be noted that a notochord (which always arises from the graft) is only formed when the organiser is taken from the centre of the dorsal lip of the blasto-pore. When taken from the side no notochord is formed though a nerve tube is induced. *Rana temporaria* and *esculenta* organisers also induce embryos in *Triton*. The converse grafts do not succeed because of the difficulty of freeing the gastrulae of *Anura* from their vitelline membranes.

The complete gastrula is the last stage from which a functional organiser can be obtained.

It must not be forgotten that while the detailed analysis of these phenomena is the work of Spemann and his school, the credit of making the first organiser graft belongs to Lewis (1907 *b*). He grafted the organiser from a gastrula of *Rana palustris* into the otic region of another embryo in the tail-bud stage, and obtained an induction of nerve tube, notochord and somites.

Brachet (1923) further investigated the properties of the organiser in *Anura*. Working on *Rana fusca* in the late blastula stage, he found that injuries (made with a needle) just below the grey crescent resulted in embryos nearly normal, while if they were above the grey crescent, the head was deficient and showed asymmetry. The malformation is however quantitative, not qualitative, the brain and neural crest are morphologically normal but too small in one region. If injuries are made in a lateral horn of the grey crescent, the trunk on that side is abnormal as regards nerve tube, notochord and somites. An interesting point to notice is that while these organs are rudimentary owing to lack of material in the injured region, in the (supposedly) uninjured region immediately behind it, they continue to be of the same abnormally small size. To explain this Brachet supposes that a formative impulse travels in a cranio-caudal direction, and that this impulse is too weak in the injured region to induce any but similarly rudimentary organs in the region immediately behind it. But this view is perhaps unnecessary when it is realised that the sides of the grey crescent (and blastopore lip) are con-stantly growing down and producing new material. One would expect therefore that an injury there would affect all regions posterior to it.

Injuries to the centre of the grey crescent result in large deficiencies as regards nerve tube, notochord and somites. Here also the notochord-forming zone appears to be restricted to the centre of the grey crescent.

Brachet concludes that the grey crescent is a region of "spontaneous" differ-entiation. "Spontaneity" merely means that the antecedent causes are unknown, except that in this case they must be connected with the entrance of the sperm.

Brachet's and Spemann's results are on the whole as similar as could be ex-pected, remembering the difference between the subclasses *Urodela* and *Anura*. The differences seem to be due to three factors (or groups of factors):

(i) Difference of experimental method, as shown by the fact that when an anuran organiser is transplanted and grafted into other *Anura* (Lewis, 1907 *b*) or into *Urodeles* (Geinitz, 1925 *a*), the results are very similar to those obtained by grafting urodele organisers into *Urodeles* (Spemann and Mangold, 1924). Injury with a needle is not as precise a method as transplanting with a micropipette, since it is difficult to be sure of the extent of the injury.

(ii) Brachet (1923) believes that in *Anura* only the anterior region of the head is preblastoporal, while in the *Urodeles* the head and trunk are preblastoporal. Goerttler's (1926) *intra vitam* markings show that the animal pole of the newt's egg becomes included behind the transverse anterior neural ridge, while that of the frog lies just in front of it. Further, in *Anura* the dorsal lip of the blastopore arises much nearer the equator than in *Urodeles*. However, the behaviour of anuran organisers in urodele tissues shows that the difference in this respect is not fundamental.

(iii) The various regions are possibly determined earlier in the frog, some at least soon after fertilisation; in the newt towards the close of gastrulation. The early specialised appearance of the presumptive organiser (grey crescent) in frogs is perhaps correlated with this accelerated *tempo* of determination. In this connection it is interesting to note that Bautzmann (1926) obtained an organiser from the early blastula stage of *Triton*.

These facts concerning the organiser immediately reveal the importance and significance of the experiments of isolating blastomeres in the 2-cell stage when it was found that only those blastomeres which contain the future dorsal lip of the blastopore would develop. This is also the explanation of the failure to develop of the ventral gastrula halves of *Triton*. It has already been mentioned that what these lack is not potency but a stimulus: the organiser. That this is so has been proved by Bautzmann (1926), who grafted an organiser into a ventral half-gastrula, and obtained the induction of an embryo.

6 ANALYSIS OF THE METHOD OF FUNCTIONING OF THE ORGANISER.

In order to explain the results which the organiser achieves, Spemann and Mangold (1924) put forward two suggestions. The effect may be due to an impulse travelling from cell to cell radiating out from the region of the organiser (a "differentiating stream"), or it may be due to the contact of the invaginated roof of the archenteron with the overlying ectoderm.

There is more than one example of supposed streams of differentiation. Braus (1906 *a*) shows something similar for the fins of sharks, and there are several examples of neuroid transmission.

Supporting the second alternative is an observation of Vogt (1922 *a*) to the effect that those embryos which lacked neural folds also lacked the roof of the gut. Geinitz (1925 *a*) removed a piece from a late gastrula of *Triton* close in front of the dorsal lip of the blastopore, and divided it tangentially into an outer (non-invaginated) and an inner (invaginated) portion which he then grafted into new hosts. He found that both portions induced a nerve tube and that in addition the former

differentiated into nerve tube and notochord, the latter differentiated into a noto-chord and mesenchyme.

Marx (1925) working on *Triton* in the late gastrula stage showed that if a piece of presumptive nerve tube were transplanted to the flank *without* any underlying gut roof being taken with it, it differentiated according to its new position into epidermis. If on the other hand some gut roof was included in the graft, it differentiated into neural folds. From earlier embryos, presumptive neural tube material always develops according to its new position whether gut roof is present or not. From older embryos, presumptive neural tube material, whether gut roof is included or not, is always self-differentiating. The only conclusion to draw is that the neural tube is determined at the late gastrula stage when the gut roof has invaginated and is intimately apposed to the underside of the presumptive nerve-tube material. Marx also calls attention to the remarkable correlation and correspondence in size which exists between the neural plate and the archenteron in embryos of several groups of Vertebrates.

This is supported by Geinitz' (1925 *a*) observation that *Bombinator* gut roof in *Triton* induces neural folds, as does *Triton* gut roof in *Triton* (Marx, 1925). Bautz-mann (1926) found that at the yolk-plug stage in *Triton*, the hinder two-thirds of the gut roof in the median and paramedian region had this capacity. He also found that, at the outset of gastrulation, the zone which was capable of functioning as an organiser coincided with that which Goerttler (1925) showed to undergo future invagination. Lehmann (1926) tested the inducing power of the gut roof by practising deficiencies at the edge of the lip of the blastopore at various stages of gastrulation. Removal at an early stage results in deficiency of the anterior gut roof and mesoderm. The overlying neural folds show a lack of lateral ridges, too thick a floor and an atypical orientation of nuclei. The neural folds are most atypical where the underlying gut roof and mesoderm is most deficient, but the correspond-ence is not so exact at the front end. The notochord is often absent and in such cases the somites fuse in the middle line; the nerve tube then shows a peculiar abnor-mality in that the floor is much too large. He concludes that the final form of the nerve tube is influenced quantitatively by the deficiency in the underlying material.

There can be little doubt after consideration of this evidence that the organiser works by pushing itself underneath the epidermis of the dorsal region and affecting it in some way. (While the nerve tube certainly is formed in this way, it will be necessary further on to consider whether it may not also have been determined before gastrulation by another means.) It explains why presumptive nerve-tube material near the blastopore is irrevocably determined sooner than that further forward, for the undergrowth takes place from behind forwards (Spemann, 1916). It explains why dorsal half-gastrulae regulate the size of their neural folds if isolated early enough (Ruud and Spemann, 1923). It explains why, by partial constriction of *Triton* embryos in the sagittal plane, Spemann (1903) obtained duplicitas anterior, since owing to the ligature the invaginated gut-roof material had to divide itself right and left and thereby induced two embryos in the anterior region; also why he never obtained duplicitas posterior by this method, since there was room

for the hinder gut roof (the "last-to-be-invaginated" material) without any necessity for it to split. Lastly it explains certain remarkable cases of duplicitas cruciata which Wessel (1926) has studied and described.

If some of the preblastoporal region of two young *Triton* gastrulae be cut off and the two gastrulae stuck together by the cut surfaces so that the blastopores gastrulate towards one another, the result varies with the distance which separates the dorsal lips of the two blastopores. If this is large the two embryos will form parallel to and facing one another on each side of a common yolk mass. The median ventral organs such as the heart and liver are then formed at the sides, and from half-components of each embryo which can be distinguished by pigment. If the distance separating the blastopores is smaller, the head ends of the two embryos will meet and turn out right and left, so that the combined neural folds form a cross. Two secondary head ends are formed in this way, each composed of tissue from each embryo. If the distance is still further decreased, the clash comes very soon, and most of the embryos are "secondary" and composite, at right angles to the original axis, and only the tails are "primary." Several points are worth noticing here: (i) formation of heads and trunks of embryos from "non-presumptive" material; (ii) alteration of axes and polarity; (iii) formation of organs at the right "level" though not in the normal axis from components of two embryos. The lack of room between the sites of invagination leads to a collision and the nerve folds deviate right and left. But the nerve folds do not arise by any process of growing forward, they arise *in situ*, and what do grow forward, collide and move to the side, are the underlying ingrowing gut roofs. This only can explain the formation of a duplicitas cruciata. Bautzmann (1926) also obtained duplicitas cruciata by organiser grafts.

In the induction of an embryo by an organiser, it is of interest to enquire into the part played by the host into which it is grafted, and the first problem which presents itself is that of orientation. Although the axis of the secondary embryo may make some angle with that of the primary host embryo, yet they never form in reversed directions, head to tail. By grafting organisers of triangular shape, and orientating them sideways or backwards with regard to the axis of the host, Geinitz (1925 *b*) found that the secondary embryo was always more or less parallel to the primary one. This points to an axial directive effect of the host tissues and of the embryo in general. Further, with regard to the position of corresponding organs in the primary and secondary embryos, it is of great interest to note that they tend to arise at the same level with regard to the animal pole, *i.e.* on the same parallel of latitude. Especially is this the case with the auditory vesicles (Spemann and Mangold, 1924; Bautzmann, 1926).

Lastly, there is the question as to what constitutes the difference between the organiser and other common pieces of tissue, in virtue of which their properties and fates differ. It is premature to speculate too much on this problem, but Geinitz (1925 *c*) has obtained some facts which furnish much food for thought. He took a piece of ordinary presumptive epidermis and grafted it into the region of the organiser so that it was carried in with the invagination; for purposes of recognition it was stained *intra vitam* or obtained from a different species. When it had been

invaginated and formed part of the gut roof it was removed and transplanted a second time into the side of another embryo, and here it proceeded to function as an organiser. This piece of ordinary epidermis had therefore been "infected" with the power to organise during its sojourn in the organiser region. This shows that the properties of the organiser are not tissue-specific in the first instance, but that they are determined by the relative position with regard to the whole embryo. This will form a subject of discussion in the section on Axial Gradients.

7. DETERMINATION AND DIFFERENTIATION IN THE BLASTODERM OF THE CHICK.

The behaviour of different regions of the chick's blastoderm at various stages of development has been tested by grafting pieces on to vascular regions of the chorio-allantoic membrane of 7-day-incubated chicks. Attempts had been previously made to obtain development *in vitro*, by McWhorter and Whipple (1912).

With *intra vitam* staining marks Wetzel (1925) showed that tissue at the posterior edge of the blastoderm stretches forward in the formation of the primitive streak. Peebles (1898) showed by means of injuries with a needle that in later development the primitive streak pushes backwards.

Danchakoff (1922) observed that the less a blastoderm has been incubated, the less growth and differentiation is obtained when it is grafted entire on to the membrane. From the advanced primitive streak stage onward, good differentiation is obtained. Kidneys, eyes, nervous system and notochord develop well.

Hoadley (1926 a) has made a close study of the powers of differentiation of isolated portions of blastoderms at different stages of development. Portions of the unincubated blastoderm will differentiate only into gut and epidermis. After 2 hours' incubation a graft will also produce the nervous system. After 4 hours, brain, eye, cartilage and muscle appear. A piece of blastoderm which has had 6 hours' incubation will after grafting show in addition ganglia, fibres, ear, glands, somites, notochord, mesonephros and heart. After 10 hours' incubation, corium and feather buds are formed. Thus it is plain that the older a blastoderm or portion of it is at the time of grafting, the more will it differentiate. This is especially well shown in the case of the eye, by grafting transverse strips of blastoderm containing the presumptive eye material. A 4-hour piece will give an eye of pigment cells only. A 6-hour piece will show differentiation into pigment and retinal cells. After 8 hours, differentiation goes as far as to show stratification of the retina. Complete self-differentiation of the eye is obtained from pieces of blastoderms which have been incubated for 33 hours. Similarly, with regard to feather buds (Hoadley, 1926 b), pieces from a blastoderm incubated for less than 10 hours will only produce the periderm and columnar layers; after 10 hours corion will be produced. In the case of the mesonephros, 4-hour pieces will give secretory tubules, 6-hour pieces also produce glomeruli, 10-hour pieces give Wolffian ducts, and if the pieces have been incubated for more than 10 hours, the entire mesonephros is differentiated (Hoadley, 1926 c). He concludes that these experiments are evidence for the existence of "preprimordial segregates," and that there is "progressive differential

dichotomy." Isolation, however, prevents further dichotomy without preventing the histogenesis and differentiation of the elements already determined by previous dichotomies. Hoadley (1926 d) has amplified these results by cutting across a blastoderm and leaving it to develop *in situ*. Here again the isolation interferes with the further morphological differentiation, without impeding the progress of the histological differentiation already reached. It is plain that the pieces are not self-differentiating until a certain stage of development has been reached; which means that they are dependent for their further differentiation on a factor situated outside them. It is tempting to compare these results with those of Hyman (1916) on the regeneration of heads from the posterior regions of the worm *Lumbriculus*. The differentiation of the regenerate into a head is governed by the size of the piece regenerating, and it is possible that a comparable state of affairs exists here. Indeed it is very probable, for Hoadley (1925 b) found that in the differentiation of the feather primordia, the larger the piece of blastoderm grafted, the less was the inhibition of the differential dichotomy, *i.e.* the more differentiation was obtained. He admits that if an *entire* blastoderm were grafted, differentiation would be complete.

From pieces of blastoderm that have been incubated for 36 to 48 hours, the rudiments of the eyes, ears and nose will develop by self-differentiation as grafts (Hoadley, 1924). Similarly, after 36 hours' incubation, somites, pronephros and neural crest (Hoadley, 1925 a). Agassiz and Danchakoff (1922) showed that, provided it is closed at the time of grafting, the neural tube will differentiate into a piece of spinal cord with grey and white matter, horns and nerves. From 7-day chicks a graft of the metanephric rudiment differentiates completely, capillaries of the allantois being induced to form glomerular knots in the concavities of the Bowman's capsules (Atterbury, 1923). Rienhoff (1922) cultivated the rudiment of the metanephros *in vitro* and obtained complete development, tubules and glomeruli differentiated *in situ*. From the 7-day chick Danchakoff (1924) found that the rudiments of the following organs would differentiate: spleen, pancreas, hypophysis, liver, kidney, adrenal, thymus, thyroid, muscle, nerve tube, heart, ovary and testis. Periosteum develops bone. She also made the very interesting observation that the mesonephros degenerates after a time in the grafts, as indeed it does in the organism.

Danchakoff (1922) grafted the anterior half of a blastoderm at the 10-somite stage, with the curious result that the pronephros was better developed than in a normal embryo. This is probably due to the removal of an inhibiting influence from the more posterior kidney elements.

Using larger pieces, Murray and Huxley (1925 a) showed that the anterior third of a 24-hour blastoderm would differentiate into a complete anterior region. The brain had the proper subdivisions, epiphysis, eyes, pituitary, lens, otic sac, grey and white matter, cranial nerves, olfactory pits, mouth, pharynx, anterior intestinal portal, thyroid, and visceral pouches were all present. It is interesting that the bulbus and ventricle only of the heart were formed. Presumably at the time of grafting the various regions of the heart were determined, and the cut

separated these from the auricle and sinus rudiments. From the middle region of a 48-hour blastoderm, spinal cord, dorsal and ventral nerve roots, vertebrae and mesonephros were formed. While there was little abnormality in the histological differentiation of the parts, their shape was often abnormal owing to the peculiar conditions of pressure and stress to which the embryo was exposed on the chorioallantoic membrane. This shows the importance of the fact that the determination of an organ and of its future histological differentiation (which is quantitative and chemical) is to be distinguished from the processes which lead to morphological differentiation (which are largely mechanical and physical, and governed by conditions of pressure and available space).

Lastly it must be mentioned that Strangeways and Fell (1926 a, b) cultivated rudiments of eyes and limbs in vitro; chorio-allantoic grafts of limbs were also studied by Murray and Huxley (1925 b) and Murray (1926); the detailed descriptions of these experiments are reserved for the special sections dealing with these organs.

This perhaps is the place in which to mention some remarkable experiments of Brachet (1912, 1913) in which he cultivated young blastodiscs of the rabbit in serum in vitro. A blastodisc aged $5\frac{1}{2}$ days doubled its diameter in 24 hours. After 48 hours it showed the primitive streak and chorionic villi. A blastodisc $6\frac{1}{4}$ days old developed amniotic folds after 24 hours, and the rudiment of the notochord was plainly visible after 44 hours. In one case, an accidental tear went through the primitive streak, and it is very interesting to note that in this case the notochord and mesoderm failed to differentiate. The self-differentiation of the villi is remarkable, showing that they are not dependent on the wall of the uterus.

8. THE NERVOUS SYSTEM.

In the late blastula stage of Urodeles, Goerttler (1925) has shown that the presumptive neural fold material is situated in a transverse narrow zone of tissue stretching across the animal hemisphere, including the animal pole and reaching down to near the equator on each side. As a result of the movements which bring about gastrulation, the side portions of this zone come to be directed backwards and are stretched along the future antero-posterior axis. The result is to bring the presumptive neural fold material into the position which these folds occupy in the neurula.

Sufficient has been said in previous chapters to show that (a) the presumptive neural fold material is not irrevocably determined until the late gastrula stage, and that (b) the organiser has the power of determining neural folds out of non-presumptive neural fold material. These facts do not however exclude the possibility that the neural folds may be determined before the late gastrula stage, a possibility expressly considered by Spemann (1918). Goerttler (1925) has tried to test this by preventing the displacement of the material from proceeding normally. This he did by making injuries or grafting foreign pieces into the region of the lateral lip of the blastopore of embryos of Pleurodeles. The result was that the presumptive neural fold material did not reach its normal destination. Yet the folds actually

formed from the presumptive material and were therefore out of place. This points to independent determination of the neural folds as regards the organiser, which, in other cases is able to induce normal neural folds. Further, Goerttler found that removal of a piece of presumptive neural fold material at the beginning of gastrulation resulted in permanent deficiency as regards the neural fold in that region, although the wound had been healed over. One would expect that as neural fold material is not irrevocably determined at this stage, and further that as the organiser can induce neural folds, the deficiency would be made good. These results are therefore in contradiction with those of Spemann and his school. Lastly, Goerttler
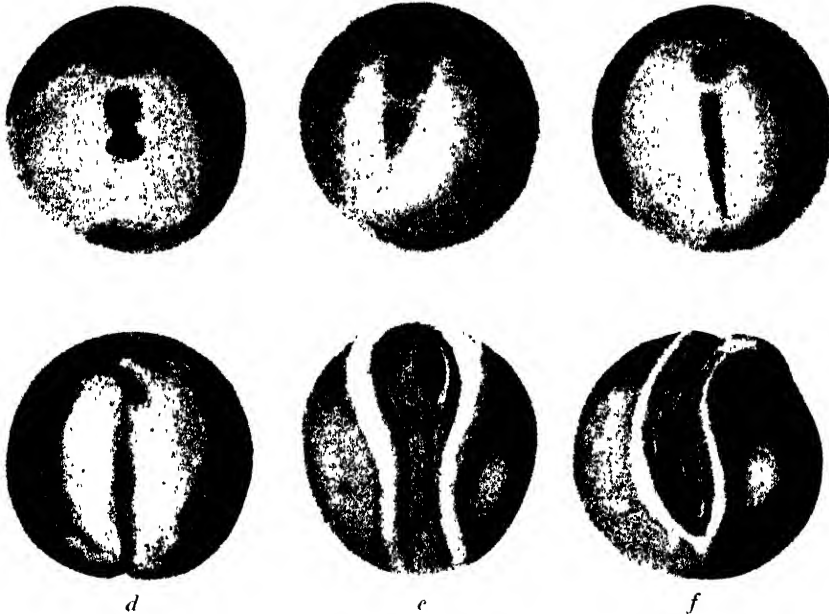
Fig. 5. Embryo of *Triton alpestris* with *intra vitam* marks in the presumptive neural fold material. Note the displacement of the lateral bands which become included within the folds, and the stretching of the tissue in the mid-dorsal line. (From Goerttler.)

(1926) removed the whole region of the dorsal lip of the blastopore at its earliest appearance in *Pleurodeles* embryos. Nevertheless, the neural folds arose from the presumptive material, which had been marked *intra vitam*. He concludes that the organiser and gut roof play no essential part in the determination of the neural folds. (It must be remembered that Lehmann (1926) found the front end of the nerve tube somewhat independent of the gut roof.) Further, Dürken (1925 *b*, 1926) obtained differentiation of a portion of a brain from a piece of the animal-pole region of a blastula of *Rana fusca*, *i.e.* a piece which had not been acted on by an organiser. This being so, the neural folds must be a structure capable of self-differentiation and also of dependent differentiation at the same time. In the course of this study several other cases will be met with of organs developing by the method of "double assurance," though in almost every case the self-differentiating property can be shown to set in after the dependent differentiating processes. In any case,

self-differentiation is at best a negative conception since it means only that at the time when it is tested, no correlation can be discovered between the structure in question and neighbouring structures. In the case of the neural folds, therefore, the possibility is open that the organiser determines them long before the dorsal lip of the blastopore appears, though the determination is not irrevocable until the late gastrula stage. In this connection it may be remembered that in the frog the organiser is foreshadowed as a very early differentiation in the form of the grey crescent, while in *Urodeles* it is not nearly so definite. This seems to be the only way of reconciling these apparently contradictory results, *i.e.* by appealing to the time factor.

If a rectangular piece is cut out of the mid-dorsal region of a gastrula of *Triton*, rotated through 180° and replaced (*i.e.* head to tail), the resulting embryo is normal as regards its nervous system, only it shows situs inversus viscerum, which will be considered later (Spemann, 1918). If the same experiment is performed at the open neural plate stage (Spemann, 1912 *b*) the nervous system is abnormal in that the rotated piece continues to differentiate as if it were still in its normal position, *i.e.* it is no longer indifferent and capable of regulation, but is determined. In such embryos the optic lobes are in front of the epiphysis and diencephalon, and the self-differentiation of regions is apparent even close up to the cut edges which have healed up with those of the surrounding normal tissue (experiments on *Rana esculenta*). (It is interesting to note in connection with the closure of the nerve tube that Giersberg (1924) has shown that it is in part a mechanical process, since the repartition of mitotic figures by itself is incapable of accounting for it. There is lateral pressure from the ectoderm as well as growth of the neural fold.)

The spinal cord of *Amblystoma* has been subjected to a very interesting analysis by Detwiler. From the medulla backwards the nerve tube tapers, so that the various segments differ in their cross-sectional area and in the number of cells and fibres they contain. If trunk segments 7, 8 and 9 of the tube (in tail-bud-stage embryos) be transplanted to the position of segments 3, 4 and 5, they attain the proper size for their new region (Detwiler, 1923 *a*). Similarly if 3, 4 and 5 be re-moved and replaced as 5, 4 and 3 (Detwiler, 1923 *b*), the original 5 behaves as if it were a normal 3 and the original 3 shows a reduction to the size of a normal 5. In this case the nerves of the brachial plexus were normal also. The next experi-ment was to remove segments 1, 2, 3, 4 and 5 and to replace them with a medulla and segments 1 and 2 from another embryo, in the proper orientation (Detwiler, 1925 *a*). The medulla occupied the regions of segments 1, 2 and 3, transplanted segments 1 and 2 were in the position of 4 and 5. It was found that these segments 1 and 2 contained more cells in the motor regions than if they had remained in their normal position. The reason for their containing more cells than normal is to be found in the increased number of impulses reaching them from the brain, which increase is itself due to the increased number of caudal projection fibres arising from the extra medulla. To prove this, segments 1, 2 and 3 were transferred to the region of 4, 5 and 6 (Detwiler, 1925 *b*). By this means 1 and 2 were in the same position as in the previous experiment, only there was no extra medulla. The
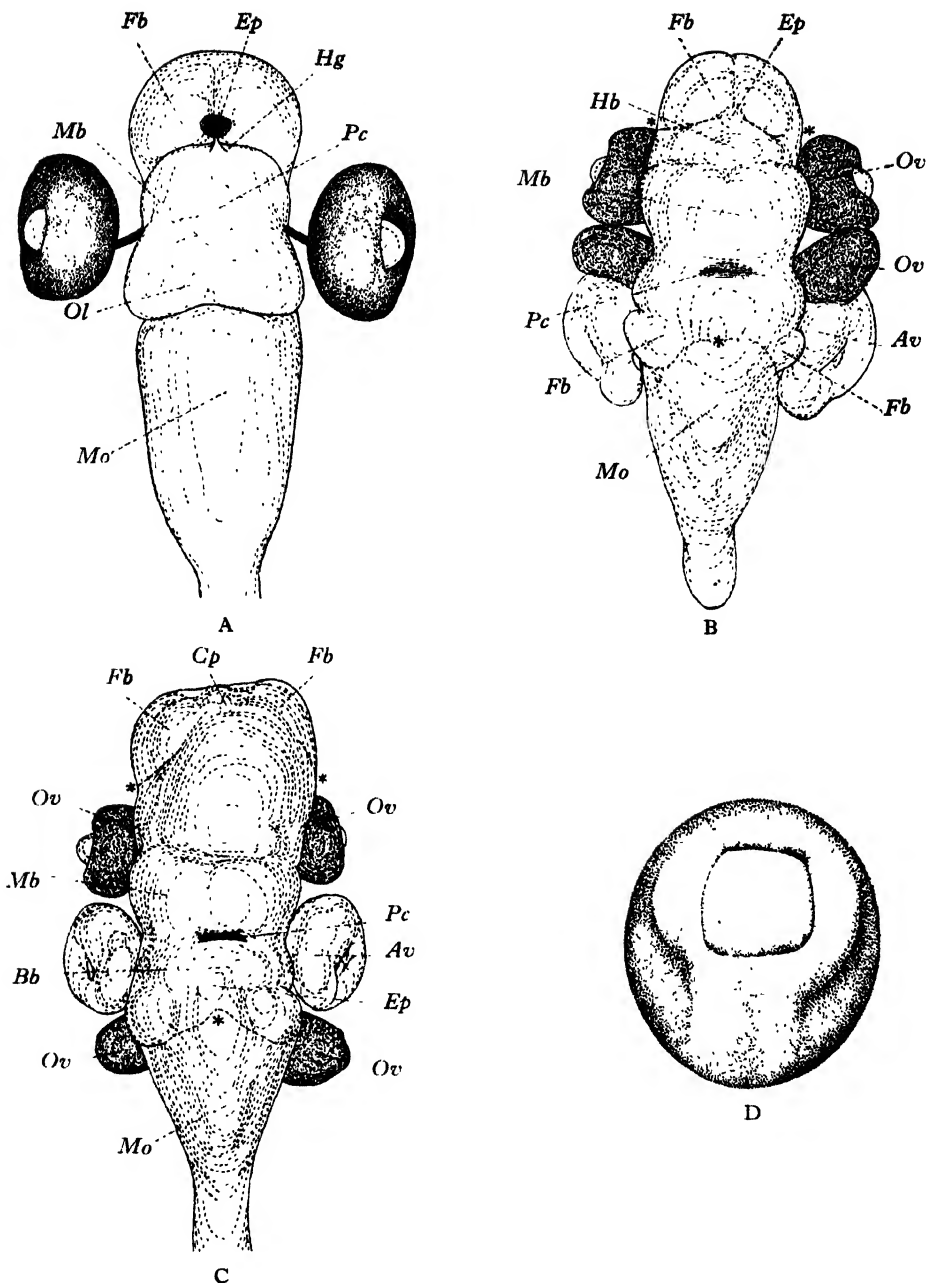
Fig. 6. A. Dorsal view of the brain of a normal embryo of *Rana esculenta*. B. Similar view of an embryo in which a short piece of the medullary plate (between the asterisks) has been rotated through 180°. Note 4 small optic vesicles. C. Similar view, after rotation of a larger piece of the medullary plate. Note 4 optic vesicles; the posterior pair behind the auditory vesicles. D. Embryo of *Rana esculenta* at the neurula stage showing the piece of the medullary plate which is rotated in these experiments.

Av, auditory vesicles; *Bb*, between-brain; *Cp*, choroid plexus; *Ep*, epiphysis; *Fb*, fore-brain; *Hb*, hind-brain; *Hg*, habenular ganglion; *Mb*, mid-brain; *Mo*, medulla oblongata; *Ol*, optic lobe; *Ov*, optic vesicle; *Pc*, posterior commissure. (From Spemann.)

result was that segments 1 and 2 maintained their normal size, which is therefore determined and self-differentiating. For the remaining segments the degree of motor cellular proliferation appears to be commensurate with the number of projection fibres. It is interesting to note in these experiments that while the nervous system is early determined as a whole, up to a certain stage it retains the power of alteration within itself, *i.e.* it is capable of regulation. After that stage it loses this power. Burr (1920) showed that at a certain stage the cerebral hemispheres of *Amblystoma* are self-differentiating, by grafting them behind the fore limb. They differentiated more or less normally, though with distortions, and reduced size owing to the lack of ascending fibre tracts.

In the newt the (motor) cellular proliferation in the nerve tube is not determined by functional activity of the peripheral musculature (Detwiler, 1924). This is shown by the fact that transplantation or removal of a limb does not affect it. On the other hand the (sensory) spinal ganglia are greatly affected by increasing or decreasing the peripheral integumentary areas. Detwiler (1926) grafted two *Amblystoma* embryos together side by side with the result that the skin area, and consequently also the spinal ganglia on the inside were much reduced. Shorey (1909) however found that in *Bufo*, *Rana pipiens* and chick, loss of a limb entailed deficiencies in the spinal cord as well as in the spinal ganglia.

Steinitz (1906) removed the eye from a frog embryo and observed a deficiency in the optic lobes. Dürken (1913, 1917) working on *Rana fusca* found that if the eye were extirpated in very early stages both the optic lobes were deficient; and the retina of the other eye was subnormal in its differentiation. If the experiment was performed later only the contra-lateral optic lobe was affected. Burr (1924) observed that after implantation of an extra olfactory organ in *Amblystoma* close to the host's own nose, there was a cellular increase of 30 per cent. in the olfactory bulb. May and Detwiler (1926), also using *Amblystoma*, grafted an extra optic vesicle behind the ear. When the optic nerve from the graft established connection with the glossopharyngeal or vagus ganglia, these showed cellular increase, and the medulla oblongata was normal. If the optic nerve entered the medulla direct, then it showed hyperplasia in its grey matter. If at an early stage a limb rudiment is removed, all the centres in the nervous system associated with limbs are under-developed (Dürken, 1912). When this experiment is performed at a later stage, if the fore limb was removed the contra-lateral centre in the mid-brain was deficient, if the hind limb, the centre on the same side. These results must be due to general debility following on lack of nervous correlation and stimulation. These experiments will be mentioned again in connection with certain peculiarities which the limbs show.

The ganglia of the cranial nerves are derived from two sources: the neural crest, and the placodes (areas of thickened epidermis). Stone (1922) attempted to show that in *Amblystoma* the cranial ganglia were largely dependent on placodes for their formation. Extirpation of the ophthalmic placode led to absence of ophthalmic ganglion and nerve. Removal of the gasserian placode resulted in deficiencies in the gasserian ganglion, and similarly in respect of the hyomandibular placode and

the facial ganglion. It is possible however that neural crest cells may also have been removed with the placode. In another experiment (Stone, 1924) the ophthalmic placode of one embryo was grafted close above the normal similar placode of another embryo. The graft gave rise to a ganglion from which fibres developed and innervated regions normally supplied by the host's own ganglion. Further, when the ophthalmic placode is grafted in the place of an extirpated gasserian placode, the ganglion so formed partly replaces the gasserian in form and nerve distribution, in particular, contributing to the mandibular nerve.

These experiments show that a certain amount of regulation takes place in nerve grafts but that their histological (chemical) differentiation is already determined. This is to be expected from Harrison's (1910) remarkable tissue cultures of nerve cells from embryos of *Rana palustris*. The cells produced axon fibres which grew out (in one case) at the rate of 56μ per hour. The longest axon thus obtained reached 1·15 mm., having taken 53 hours to grow. Ingvar (1919) carried the analysis a stage further by showing the reaction of these axons to electric currents. The fibres grow out along the lines of the field of force of a very weak current. If however a conductor goes through the culture and a current passes through it, the fibres grow out at right angles to this conductor. Now it has been shown in many animals (see Hyman and Bellamy, 1922, and Child, 1924) that a difference of potential exists between the anterior and posterior ends of bilaterally symmetrical animals. Consequently it can be understood why fibres grow up and down the nerve tube and also, since when impulses travel through them these fibres act as conductors, why other fibres then grow out at right angles to them, as do the spinal nerves.

This is perhaps the place in which to consider the question of the outgrowth of nerve fibres to their end organs. Harrison (1910) showed that they could grow out freely *in vitro*. Hoadley (1925 *b*) found that when a portion of the brain and of the trunk region of a chick embryo are grafted together on to the chorio-allantoic membrane, nerve fibres develop and enter and innervate various structures. On the other hand, if only mesenchyme is present without other structures, the nerve fibres will not grow out far. The evidence is therefore all against the theory of pre-established connection between nerve and end organ, and this is proved conclusively by the following experiment. Harrison (1907) removed the nerve tube from an *Amblystoma* embryo and thereby destroyed all possible connection between nerves and limb buds. He next transplanted these "aneurogenic" limbs into the normal region of another embryo, where they became innervated in the normal manner. The nerves must therefore have grown out freely. Lewis' (1907 *c*) results also show that nerves can grow out freely. Detwiler (1920, 1922) grafted arm buds of *Amblystoma* into regions slightly distant from normal, and found that they still become innervated by the proper nerves from the normal level of the spinal cord provided that they are not too far distant. There must therefore be some chemotactic attraction of the nerves to the limb. If they are too far distant, their supply is abnormal. Similarly when the region of the nerve tube associated with the brachial plexus is rotated 180° (Detwiler, 1923 *b*), the brachial

plexus is identical with the normal. Transplanted limbs may function perfectly even when grafted from *Amblystoma punctatum* to *A. tigrinum* (Harrison, 1924), and when the spinal cord of *punctatum* in the arm region is exchanged for that of *tigrinum* (Wieman, 1926).

The organs of the lateral line were among the first to be studied. Harrison made use of Born's (1897) method of grafting together two portions of embryos belonging to different species. By grafting the head end of the dark-coloured embryo of *Rana sylvatica* on to the lighter coloured trunk of *Rana palustris* he was able to see that the lateral line grew back from the dark portion over the lighter (Harrison, 1904). It grew back at the proper level with regard to the side even when an intermediate piece of tissue over which it had to travel was rotated 180° either in the vertical or in the antero-posterior axis. Also when the head was grafted at an angle of 90° to the tail, the lateral line grew down till it reached the proper level and then grew back along it. Its path is therefore in some way governed; yet not absolutely rigorously, for if a scar was in its track, the line grew round it. This experiment is of the greatest interest in connection with Axial Gradients, when dealing with which it will be mentioned again.

Stone (1922) removed the vagus placode from embryos of *Amblystoma* and found that the lateral line did not develop; similarly extirpation of the preauditory placode resulted in deficiencies in the supra-orbital line.

In other experiments on *Amblystoma*, Stone (1925) planted the postauditory (vagal) lateral line placode in the place of the preauditory (facial) placode. It formed a definite supra-orbital line along the correct pathway, and the associated ganglion and nerve simulated the normal. When the facial placode was planted in the place of the vagal, it formed a lateral line which grew back in the proper place (although the placode had been rotated through 180°) but did not exceed the length which it would have reached in the normal (supra-orbital) position.

9. THE EYE, THE LENS AND THE CORNEA.

In Spemann's (1912 *b*) experiments already mentioned, in which a rectangular mid-dorsal piece was rotated at the open neural fold stage of *Rana esculenta*, the anterior cut edge usually went through the rudiments of the eyes, so that part of them were left in place, and part found themselves at the hinder end of the rectangular piece as a result of the rotation. This is proved by the fact that while a pair of small eyes were found in front in the normal position, another pair were formed behind, in front of or behind the ear vesicles according to the length of the rotated piece. (See fig. 6.) This shows in the first place that the eye rudiments were determined since they differentiated independently in strange surroundings. Further, since the sum of the sizes of the right fore and left hind eyes equalled that of the left fore and right hind, this determination was quantitative. When such eyes were very small, they had arisen from only a small part of the eye rudiment, which might consist only of tapetum pigment cells. Other eyes had no tapetum, and the rods and cones of the retina projected freely into the cavity of the brain: others again had too much tapetum, and this was thicker than normal. This means that the

various constituent parts of the eye are also qualitatively and quantitatively determined ("chemo-differentiated," Huxley, 1924) in the open neural fold stage; and the cuts in the experiment separated these constituents and distributed them unequally in the various cases. This was also the conclusion to which Lewis (1908) came when he transplanted the (still-invisible) presumptive eye rudiment of *Rana palustris* in the open neural fold stage. There were variations in the ganglionic layer, pigment layer, etc., which can be explained by an invisible determination of the various constituents at the time of transplantation. At the same time it is worth noticing that however deficient the eye is (within limits) it undergoes the morphological differentiation and regulation which makes it resemble a cup. Morphological and histological differentiation are therefore sharply distinct processes. Filatow (1926) cultured *in vitro* a portion of the eye rudiment of *Rana esculenta*. Both this piece and the piece left in the embryo regulated to form little eye cups. Similarly two eye cups in contact tend to regulate to one (Anastasi, 1913).

That the eye rudiment is self-differentiating at this stage, there is then no doubt. The most striking demonstration of this is Spemann's (1925) experiment in which he grafted the presumptive eye rudiment of a neurula of *Bombinator* into the flank of another. It developed in the body wall beneath the pronephros into an optic cup with the concavity towards the coelom. Other grafts in *Amblystoma* were made by May and Detwiler (1926). Filatow (1926) cultured *in vitro* the eye rudiment of *Amblystoma*. If taken from the open neural fold stage he did not succeed in getting it to.differentiate. On the other hand, if taken from the stage at which the neural folds are first closed, it differentiates properly with a lens, almost keeping pace with the normal eye. Werber (1915) subjected embryos of *Fundulus* to acetone, and, among other abnormalities, obtained "meroplastic" embryos, in which only certain regions developed at all. In some cases this was a fragment of the neural plate, which developed by self-differentiation into a solitary isolated eye.

Strangeways and Fell (1926 b) grew the eye of the 64-hour chick in tissue culture, at which stage it consists of an outer layer one cell thick and an inner layer of epithelial cells. *In vitro* it differentiated into pigment, rods and cones, inner and outer nuclear layers, inner and outer plexiform layers, ganglionic cells and nerve fibres. Hoadley (1924) obtained differentiation of the eye of a 33-hour chick on the chorio-allantoic membrane.

Mention must now be made of some experiments in which Stockard (1910) subjected embryos of *Fundulus* to various solutions, such as alcohol, chloroform, ether and magnesium chloride, and obtained various degrees of fusion of the two eyes, a condition known as cyclopia. The median and intervening portions of the head appear to be missing, and only the lateral parts are well formed, though displaced towards the middle line to meet those of the opposite side. McClendon (1912) emphasised the fact that these results are not due to any specific action of the individual chemical compounds. He obtained cyclopia by using KCl, NaCl, NaOH, and cane-sugar. In the frog *Rana pipiens* Bellamy (1919) obtained fusion of the eyes, nasal pits, and ventral suckers by subjecting the embryos to LiCl.

Cotronei (1921 c) also treated embryos of *Rana esculenta, Bufo vulgaris* and *Triton cristatus* with LiCl, and obtained cyclopia, often accompanied by other abnormalities as regards the nose and mouth. In some cases in *Rana* and *Bufo*, he obtained anophthalmia. These results are comparable with those which Child (1924) has obtained in *Planaria*.

This raises an interesting question. In development, are the eye rudiments first of all median, only moving to the side later? If so Stockard's results are due to prevention of this lateral movement. Or are they from the beginning paired? In which case Stockard's cyclopic fish are to be explained by a deficiency of median material. Stockard (1913) tried to test this on *Amblystoma* material at the open neural fold stage. When lateral regions of the rudiment were removed 80 per cent. of normal eyes were obtained, while removal of median regions showed 48 per cent. without eyes. He therefore concluded in favour of the first alternative. Against this must be set the fact that Lewis (1909) by making a median injury in the embryonic shield of *Fundulus* obtained cyclopic embryos. Here clearly the result is due to median deficiency, not prevention of lateral movement. Further, Fischel, (1921) as the result of considering a series of cyclopic malformations in *Salamandra maculosa*, observed that the optic malformation was proportional to the nasal. He concluded that this parallel monorhiny would be inexplicable if cyclopia were due to non-separation of median eye rudiments, but must be due to median deficiency between paired rudiments.

Meanwhile, it may be noticed that in Spemann's (1921) experiments, in which a piece of strange presumptive epidermis was transplanted to the anterior presumptive neural fold region, the eye rudiment developed *in situ*; there was no movement away from the middle line. This result is conclusive. In other cases, Spemann (1904) found that one head of the two forming part of a duplicitas anterior might have malformations leading to and including cyclopia; and that this occurred when the plane of constriction caused by the noose of the ligature was slightly oblique with regard to the original plane of bilateral symmetry. One half was therefore slightly deficient in anterior material, leading to cyclopia.

A difficulty arises here. In Stockard's experiments the exposure of the embryos to the solutions causing cyclopia was at a time long before the determination of the rudiments of the eye (as proved by other experiments). How then can a deficiency be brought about in a rudiment which has not been formed? It may be noticed that the deficient region comprises what is morphologically the most anterior point of the embryo; *i.e.* that region which on the theory of Axial Gradients has the highest rate of protoplasmic activity. This region presumably is the first to suffer from the solutions in question, with the result that its relative rate is no longer maintained. Since there is reason to believe that qualitative differentiation depends partly on quantitative rate of protoplasmic activity (see p. 189), it is easy to see that suppression or rather lowering of the relative rate in the region where it is normally highest will result in the non-formation of the structures which would normally arise there.

This explanation will also account for Spemann's cases just mentioned. The

deficient half-embryo (it must be remembered that the embryo is *not completely* constricted into two, but the term "half" is used for facilitating description) lacks the most anterior region: the highest point of the gradient. At the same time, where the two heads join on to the common trunk is a region of definite rate which is the same for both. The "potential difference" in rate along the gradient between this point and the front end of the head which possesses the region of highest rate is sufficient to give rise to the normal structures there. But in the case of the other head which lacks the region of highest rate, the "potential difference" is insufficient, and cyclopia results for the same reason as in Stockard's experiments. The suggestion that these abnormalities (in Vertebrates) could be explained by means of Axial Gradients is due to Werber (1915) and Newman (1917).

The invagination of the optic vesicle to form a cup must be a self-differentiation. Ekman (1914 *b*) observed a case in *Rana esculenta* and in *Bombinator* in which the eye had two inpushings. Similar cases were found in *Salamandra* by Fessler (1920). Cotronei (1921 *a*) has shown that these apparent duplicities (which he obtained experimentally in *Bufo vulgaris*) are the result of mechanical conditions of available space and cell division inside the optic cup. They are in·fact an exaggeration of a condition through which, according to Rabl (1917), the eye passes in its normal development in all Vertebrates.

It is now time to turn to the developmental mechanics of the lens, and as a prelude it is only necessary to stress the fact that although the eye cup and lens become so perfectly adapted to one another later on, they arise from quite separate rudiments, the lens being epidermal. It will be convenient to deal with the different species operated upon in turn.

Rana fusca seu temporaria. Spemann (1901 *b*) destroyed the eye rudiment at the open neural fold stage and found that although the presumptive lens tissue had not been touched, no lens developed. Filatow (1926) grafted the eye under the skin in the trunk region and obtained differentiation of a lens and he also (1924) obtained a lens from strange ectoderm over the eye *in situ*. Jenkinson (1906) subjected developing embryos to solutions of NaCl, NaBr, or $NaNO_3$, whereby the eye cups remained deep beneath the surface, and no lenses formed. On the other hand von Ubisch (1925) found that after removal of the eye a small "lentoid" might be formed, also after partial inhibition of eye development with Na_2CO_3.

Hyla arborea. Ekman (1914 *a*) grafted epithelium from a distance over the eye in the proper place, and obtained a lens. On the other hand the presumptive lens epithelium transplanted to other regions gave no lens.

Chick. Danchakoff (1924) grew a piece of the anterior region of a blastoderm at the head-process stage as a chorio-allantoic graft, and found that an eye cup differentiated and that a lens could be formed from strange epidermis, and perhaps from chorionic ectoderm.

Rana sylvatica. Lewis (1904, 1907 *a*) removed the eye and grafted it under the skin of the trunk, and obtained a lens.

Pelobates. Wachs (1920) found that after removal of the lens, the eye produced another from the margin of its cup.

Bufo vulgaris. Cotronei (1921 *a*) observed that in cases treated with LiCl the eye cup did not touch the skin, and the lens was not formed.

Filatow (1924) grafted non-presumptive lens epidermis over the eye and obtained a lens, also when it was grafted over the eye of *Rana esculenta*.

Triton taeniatus. Spemann (1905) removed the presumptive lens epithelium, and the gap closed by approximation of the sides of the wound. A lens was formed, therefore, from strange epidermis. But if the epidermis and the eye cup were separated by connective tissue, the lens was formed from the edge of the eye cup and not from the epidermis. Wachs (1914) also obtained a lens from strange epidermis.

Rana palustris. Lewis (1904, 1907 *a*) removed the eye and got no lens. King (1905) however found that a lens might be formed under these circumstances, but it was small. Strange epidermis over the eye produced a lens, even if it came from *Rana sylvatica*; and the eye grafted under the skin of the trunk induced a lens.

Bombinator pachypus. Spemann (1912 *a*) showed that removal of the eye might not prevent a large lentoid or slightly deficient lens from developing. Strange epithelium from the head grafted over the eye produced a lens of more or less normal size, but no lens could be obtained from epithelium from the trunk.

Amblystoma punctatum. Le Cron (1906) removed the eye at early stages and got no lens; on the other hand at later stages the lens might develop but was subsequently resorbed. Harrison (1919) showed that if the presumptive lens epidermis was removed, a lens formed from the regenerated tissue closing the wound, which has been in the immediate vicinity. On the other hand more distant epidermis grafted over the eye gave no lens. Presumptive lens epithelium grafted elsewhere before the neural folds close gives no lens. If it is taken after the closure of the neural folds, a lens does form. This is remarkable since extirpation of the eye at this stage prevents lens formation.

Rana arvalis. Filatow (1925) found that a lens would not develop from strange epidermis.

Rana esculenta. Removal of the eye does not prevent the formation of the lens, although it may be smaller than normal (Spemann, 1907 *a*). No other epidermis region grafted over the eye will give a lens (Spemann, 1912 *a*). Yet trunk epithelium of *Bufo vulgaris* grafted over the eye does give a lens (Filatow, 1925), and the eye of *esculenta* grafted under the skin of the trunk of *Bombinator* gives a lens (Spemann, 1908). Transplantation of the presumptive lens tissue may give rise to a lens (Spemann, 1912 *b*), but in these experiments (rotation of a rectangular mid-dorsal piece) it is noteworthy that no lens formed at the proper place if the eye were too small or did not touch the skin. In other cases the size of the lens was adapted to that of the eye.

Salmo. Mencl (1903) observed cases without eyes and in which lenses were nevertheless present.

Fundulus. Stockard (1910 *b*) induced artificial cyclopia, and found that the lens did not always conform to the eye. On the other hand when the lens was median

and single it probably arose from strange tissue, which led Herbst (1901) to suspect the correlation in development between eye cup and lens.

To all these must be added the fact that in *Rana fusca* (Bell, 1906), *Rana esculenta* (*ibid.*) and *Rana sylvatica* (Lewis 1904) the lens may be formed from the edge of the iris, as in certain cases of regeneration. It may be convenient to set out these results in tabular form (see p. 168).

Stockard (1907) has shown that in the development of *Bdellostoma*, contact between the eye cup and the epidermis results in the formation of a lens. Later, however, this contact is lost and the lens degenerates.

From all this it would appear that in one set of species the lens was dependent for its differentiation on the eye (*Rana fusca, sylvatica*; *Hyla arborea*; *Bufo vulgaris*; *Triton taeniatus*; *Amblystoma*); in another set independent (*Rana esculenta, arvalis*; *Fundulus* and *Salmo*) and self-differentiating.

It is obvious, as Spemann (1907 b) pointed out, that the various species of one genus cannot have radically different methods of forming the lens. There must therefore be some relation between self- and dependent-differentiation. In the first place it must be observed that the eye is in all cases capable of inducing a lens, for while in *esculenta* it cannot do so from its own strange tissue, it can from that of *Bufo* and *Bombinator*. The non-formation of a lens in *Rana esculenta* from strange epidermis grafted over the eye is therefore due to specialisation of the epidermis. In this connection it has been proved in *Amblystoma* and *Bombinator* that distant tissue will not produce a lens, while tissue nearer to the presumptive lens-site will. Now with regard to the power of a lens to self-differentiate it is to be noted that *Rana palustris* and *Bombinator* occupy an intermediate position, in fact even *Rana fusca* can sometimes produce a lentoid. Further, the experiments on *Amblystoma* show that the power of self-differentiation increases with age.

It may be concluded therefore that in all the amphibia at least, the lens is at some time dependent in its determination, but that in some groups this chemo-differentiation may occur very early, in the open neural fold stage (*Rana esculenta*), in others very late (*Rana fusca*). In all cases, however, contact of the eye with the skin appears to have some effect. Hoadley (1926 d) has made the interesting observation that in certain experiments on the chick, the lens is induced not by the optic cup (which has not yet been formed) but by the optic vesicle. This means that the inducing factor is independent of the degree of *morphological* differentiation reached by the eye, and must be connected with its *histological* or *chemical* degree of differentiation.

Von Ubisch (1922) has suggested that these differences can be explained with reference to temperature, since *Rana fusca* develops slowly (in cold weather), *Rana esculenta* rapidly (at a warmer time of year). Experiments show however (von Ubisch, 1924, 1925) that the self-differentiation of the lens is impeded in *Rana esculenta* at low temperature, in *Bombinator* at high temperature, while that of *Rana fusca* is more or less independent of temperature.

The differentiation and clearing of the cornea and conjunctiva must be considered before leaving the eye.

	Eye absent, removed or too deep	Strange epidermis over the eye	Eye grafted under strange epidermis	Presumptive lens material transplanted	Lens formed from edge of eye
Rana fusca	No lens, Spemann. No lens, Jenkinson. Lentoid, von Ubisch	Lens formed, Filatow	Lens formed, Filatow	—	Bell
Hyla arborea	—	Lens formed, Ekman	—	No lens, Ekman	—
Chick	—	—	Lens formed, Danchakoff	—	—
Rana sylvatica	—	—	Lens formed, Lewis	—	Lewis
Bufo vulgaris	No lens, Cotronei	Lens formed, Filatow	—	—	—
Triton taeniatus	—	Lens formed, Spemann, Wachs	—	—	Spemann
Rana palustris	No lens, Lewis. Lentoid, King	Lens formed, Lewis; also from *R. sylvatica*	Lens formed, Lewis	—	—
Bombinator pachypus	Lentoid, Spemann	Lens from head, no lens from trunk, Spemann	—	—	—
Amblystoma	No lens, Le Cron	Lens from near, no lens from far, Harrison	—	No lens if early, lens formed if late, Harrison	—
Rana esculenta	Lens formed, Spemann. Sometimes no lens, Spemann	No lens, Spemann. Lens formed from *Bufo* skin, Filatow	Lens formed in *Bombinator*, Spemann	Lens formed, Spemann	Filatow, Bell
Rana arvalis	—	No lens, Filatow	—	—	—
Salmo	Lens formed, Mencl	—	—	—	—
Fundulus	Lens formed, Stockard	—	—	—	—
Pelobates	—	—	—	—	Wachs

Lewis (1905), working on *Amblystoma* and *Rana sylvatica*, showed that early removal of the eye resulted in no cornea being differentiated. Spemann (1901 *b*) came to the same conclusion with regard to *Rana fusca* after removing the presumptive eye rudiment at the neural plate stage. Dürken (1913) also observed absence of conjunctival clearing in *Rana fusca* after removing the optic vesicle from a later stage. If the eye and lens are removed after the lens has separated from the skin, a small corneal area develops. The size of the cornea is adapted to the size of the eye when the latter *is* experimentally reduced. The eye can form a cornea in the absence of a lens. If the eye is removed soon after the lens is formed and the lens left, a small cornea is formed. Further, if the presumptive corneal epidermis is removed, a cornea can develop from the regenerated tissue. This result was obtained by Groll (1924) in *Rana fusca* also, when distant skin was grafted. He confirmed Dürken's (1913) observations that extirpation of the eye did not hinder the development of eyelids. Furthermore, he found after having differentiated, the cornea became opaque again if the eye was removed. The cornea therefore seems to require the eye not only for differentiation but also for maintenance. However, Luther (1916) removed the eye of *Rana fusca* and observed that nevertheless the cornea developed. Cole (1922) obtained interesting results from strange skin grafted over the eye in *Rana clamitans* and *catesbeyana*. In 60 per cent. of cases in which tail skin was grafted, there ensued a perforation and absorption, thereby exposing the eye. This absorption also takes place if tail skin is grafted over an artificial "eye" in the shape of a glass bead, showing that it is the result of the mechanical pressure caused by the curvature. These results are not obtained with skin from the back.

Fischel (1917) grafted the lens in *Triton* under the skin of the back. The gland cells disappeared, the area cleared and became a two-layered epithelium strongly reminiscent of a cornea.

Dürken (1916) extirpated the eye of *Rana fusca* and grafted a limb bud into the orbit. The conjunctiva became clear, which without the limb or the eye it would not have done. Here again, pressure appears to be the determining factor.

It is regrettable that authors have not always been precise in their usage of the terms cornea and conjunctiva. Lewis' (1905) paper is entitled "On the cornea," yet Groll (1924) refers to it as on the conjunctiva, as does Dürken (1916). Lewis himself (1905, p. 431) refers to "cornea or rather corneal changes of the ectoderm." At all events, it seems that the results described above apply to both the cornea and the conjunctiva *sensu stricto*.

10. THE EAR.

In some of Spemann's (1902) experiments on *Triton* in the open neural fold stage, it was found after transverse division that both portions of the embryo which developed contained small otic vesicles. The division must therefore have passed through the rudiments of the otic vesicles. Levy (1906) removed portions of the anterior regions in *Triton* embryos and obtained reduced otic vesicles. These experiments show that at this stage the otic vesicle is already determined. Further,

it is incapable of regulation, for Spemann (1910) divided a neurula of *Rana esculenta* transversely and found that while both portions contained rudimentary vesicles they were incomplete, since only one on each side had a ductus endolymphaticus. The various parts of the vesicle are therefore also determined. This is further supported by Streeter's (1907) discovery that the ductus endolymphaticus might be histologically normal when the rest of the vesicle was not. Sternberg (1924) grafted the vesicle of *Rana fusca* into the ventral gill region and observed self-differentiation of the ganglia and sense organs. Lewis (1906) grafted the vesicle of *Rana palustris* into *Amblystoma* and obtained self-differentiation. Hoadley (1924) obtained the same in the chick when the rudiment was grafted on to the chorio-allantoic membrane. Streeter (1909) found that two vesicles grafted together in *Rana* did not regulate into one. Eisinger and Sternberg (1924) removed the vesicle of *Rana fusca* and found that it was not regenerated. There is therefore no doubt that the otic vesicle at and after the neurula stage is self-differentiating and is further not an "equipotential harmonic system" (Driesch, 1921) but a "mosaic."

For facilitating the description of experiments, Harrison (1921 *b*) and Milojewic (1924) have introduced a terminology which may conveniently be described here.

Homopleural and Heteropleural denote whether the graft is planted on the side of its own origin, or on the opposite side.

Dorso-dorsal and Dorso-ventral indicate whether the dorso-ventral axis of the graft has been reversed in the experiment.

Antero-anterior and Antero-posterior give similar information with regard to the antero-posterior axis of the graft.

Medio-medial and Medio-lateral refer to the orientation of the medio-lateral axis of the graft.

A graft is Orthotopic if it is planted into a region similar or identical to that whence it was taken, if the site of implantation is different the graft is Heterotopic. In the case of limbs, an anterior limb graft in the region of the anterior limb is Homonomic, similarly a posterior limb graft in the region of the posterior limb. An anterior limb graft on the site of a posterior limb, or *vice versa*, is Heteronomic.

Turning now to earlier stages in the differentiation of the ear, Tokura (1925) found that in *Rana nigromaculata*, at the stage when the otic rudiment is just a thickening, rotation of this rudiment (*i.e.* homopleural dorso-ventral graft) leads to development in the reversed position, and also that a left rudiment on the right side (heteropleural) maintains its laterality. He further observed that if, at the stage before the otic thickening appears, the presumptive otic region is removed, a normal though smaller vesicle is formed from the neighbouring epidermis which has closed over the wound. This occurred in 17 out of 25 cases while the neural folds were still open. On the other hand, after the neural folds had closed an abnormally small vesicle was found, and only in 3 out of 25 cases.

In *Amblystoma*, Kaan (1926) has shown that before the otic cup is invaginated, the ectoderm for a considerable region round the site is capable of forming a vesicle, or even two more or less normal vesicles. After the otic cup has been formed, this power is lost by the other regions of the skin.

In order to ascertain what were the conditions in relation to the determination of the ear, Lewis (1906) removed the presumptive otic epidermal region and planted it back again, thereby destroying any connections with the underlying material. He also removed the cranial ganglia, or even the side of the brain, but in all these cases normal vesicles arose (in *Rana palustris*). Tokura (1925) also removed the side of the brain in *Bufo japonicus*, with the same result. It has not been possible therefore, at these comparatively late stages, to find a structure on which the otic vesicle is dependent for its determination, but it must not be forgotten that in an earlier stage grafts of organisers have the power to induce the formation of otic vesicles which otherwise would never have existed. Further, there must be some form of determination of the otic vesicle in the blastula, as shown by Dürken (1925 *b*).

Some very interesting results have been obtained by rotating vesicles through the various axes, and transplanting those from one side to the other.

Streeter (1906) transplanted the vesicle of the left side into the normal position on the right side in the frog. The result was that the vesicle maintained its laterality, but otherwise it assumed the correct positions, *i.e.* median side towards the brain, and dorsal side upwards; only the lagena pointed forwards instead of backwards, and the anterior semicircular canal projected caudally. Tokura (1924) found the same for *Rana nigromaculata*, which is indeed what would be expected from a self-differentiating organ. But the curious thing is that the dorso-ventral and medio-lateral axes should be correct, even when the graft had been heteropleural dorso-ventral. This is especially curious in cases where vesicles are rotated and left to develop on their proper side. Streeter (1914) found that if a vesicle were turned upside down and median side out it nevertheless tended to right itself (*Rana pipiens*). Ogawa (1921) found the same for *Rana palustris*, and for *Bufo japonicus* (Ogawa, 1922). Spemann (1910), however, rotated vesicles of *Rana esculenta* 180° about the transverse axis, and in most cases they developed in the rotated position with the ductus endolymphaticus pointing down instead of up. In one case the vesicle slipped back into the correct position. In *Rana nigromaculata*, Ogawa (1921) found that in 5 cases out of 9 the transversely rotated vesicle righted itself. Further (1926) he found that a half vesicle would right itself. This power decreases with age, and he was able to show that it is the age of the vesicle and not that of the surrounding tissues which limits the power, for rotation takes place when a young vesicle is grafted into an older larva.

This position regulation cannot be explained by the assumption that the ear is a harmonic equipotential system, for the many demonstrations of mosaic self-differentiation prove that it is not. It must therefore be due to a reverse rotation of the whole organ. This is not due to the relations of nerves to the vesicle, since Ogawa (1921) has shown that the righting takes place even when the rotation was performed at an early stage, before any nerves grew out. The suggestion that the ear rights itself because it only fits properly one way round in the neighbouring structures is disproved by the fact that a right vesicle adopts the "correct" position on the left side, although thereby there is a misfit between the lagena and the space

which normally accommodates it, and also because Ogawa (1921) has shown that *Rana* vesicles grafted into *Amblystoma*, and *Amblystoma* vesicles into *Rana*, can right themselves. In all these cases the rotation is gradual, and the cases of non-rotation and of retention of laterality show that the axes are fully determined in the rudiment. It is possible therefore that the vesicle rotates into the correct position either in relation to gradients in the organism, or in relation to gravitational stimuli. In this connection it is interesting to notice that the cases in which rotation of the vesicle is performed, and no righting occurs, lead to tadpoles which swim upside down and in all sorts of abnormal ways (Spemann, 1906 *a*). It is of interest to compare these processes of rotation in the ear with those which take place in the limb and girdle (see section 13). By a process comparable to that which gives rise to cyclopia, fusion of the auditory vesicles can be obtained under experimental conditions which lower the rate of protoplasmic activity at the anterior end of the organism (Reagan, MacMorland and Mudd, 1917). The power of the otic vesicle to induce the cartilaginous capsule is dealt with in the section on the skull, its power to induce a limb, in the section on limbs.

11. THE NOSE.

Ekman (1923) has shown that in *Rana fusca* the rudiment of the nose can be formed from tissue which has grown over to cover the wound caused by removal of the presumptive nose tissue; but more distant skin will not do so. Bell (1906) extirpated the nose rudiment in *Rana esculenta* and obtained the same result. In one experiment (Bell, 1907 *a*) he grafted the presumptive nose rudiment over the eye, in a 3·5 mm. embryo, where it self-differentiated. Lewis (1907 *c*) grafted the nose rudiment of *Amblystoma* just dorsal to the eye in another embryo. Differentiation continued and nerve fibres were given off. At the same time, in the correct position, a small nose rudiment was formed from the tissue which had closed over the wound. Hoadley (1924) obtained self-differentiation of the nose rudiment grafted on to the chorio-allantoic membrane. May and Detwiler (1926) showed that transplanted nose rudiments could self-differentiate in *Amblystoma*, while Spemann (1912 *b*) got the same result in *Rana esculenta*. In *Amblystoma* also, at the 5-mm. stage, Burr (1916 *a*) showed that after extirpation of the nose rudiment the neighbouring tissue could not form it. He also showed (Burr, 1916 *b*) that the nose is necessary for the regeneration of the fore brain. In Ekman's (1923) experiments on *Rana fusca*, it was found that however small and deficient the nose rudiment might be, if it reached the mouth epithelium at all, the latter responded by differentiating typical choanae. The latter are therefore dependent on the nose for their differentiation.

The relations of the nose to the olfactory cartilaginous capsule and to the mouth will be dealt with in the sections 14 and 16. The effects on cellular proliferation in the brain of grafting an extra olfactory organ on the head (Burr, 1924; May and Detwiler, 1926) have been dealt with in section 8.

12. THE PITUITARY.

Smith (1920) extirpated the hypophysis with knives from embryos of *Rana boylei* and *Bufo boreas*, 3½ to 4 mm. long, in the tail-bud stage. When the removal was complete, great changes were found in the infundibular constituents of the pituitary. Instead of showing the characteristic neuroglial thickenings, the infundibular floor was thin and membranous, the pars nervosa was diminished in size by 40 to 80 per cent., and was oval instead of being dumbbell-shaped.

When the removal of the hypophysis was incomplete, the remaining diminished portion of it might establish contact with the infundibulum in front of its normal position. It is noteworthy that in these cases the adjacent regions of the infundibular floor became thickened. The hypophysial portion did not however differentiate into pars anterior and pars intermedia unless it reached its normal position.

The neural elements of the pituitary are therefore dependent on the hypophysial for their proper differentiation, and the hypophysial are dependent on proper contact with the neural for theirs. It must be remembered, however, that in Mammals (Holt, 1921) a case is known in which the neural elements were properly differentiated in the absence of the hypophysial.

13. THE LIMBS AND GIRDLES.

To Braus (1905) belongs the credit of testing the powers of differentiation of the limb bud by grafting and transplanting.

Detwiler (1918) found that, in *Amblystoma*, the fore-limb rudiments are already determined at the neurula stage, and when transplanted they continue their development by self-differentiation. Harrison (1918) showed that the rudiment zone was in the form of a circle extending from the third somite to the middle of the sixth, and situated just beneath the pronephros. A normal limb can be formed from half a rudiment and from two rudiments fused together, so that it is a harmonic equipotential system. The cells round the rudiment have the power to form a limb, but with diminishing intensity as distance increases. Swett (1923), by means of an ingenious and laborious *intra vitam* staining method, was able to show that all the cells capable of limb formation did not take part in the production of a limb in normal development. By grafting the mesodermal tissues of the presumptive rudiment apart from their ectodermal covering, Harrison (1918) proved that the mesoderm is responsible for determining a limb, and Detwiler (1922) obtained normal limbs after grafting strange ectoderm over the presumptive limb region. The cells in the dorso-anterior quadrant of the rudiment have the greatest potency for limb formation (Harrison, 1918).

Great interest attaches to certain other experiments of Detwiler (1920) in which the limb bud was transplanted to other regions on the side at varying distances from the normal position. The cells round the original position gave rise to a small limb, but the degree of development which it achieved varied directly with the distance which separated it from the transplanted limb. The latter larger limb inhibits

the other if it is too near to it; *i.e.* within a certain range in which it is dominant. (See Child (1924) for other examples of physiological dominance.)

Attention must now be paid to certain experiments of Harrison (1921 *b*, 1925 *b*) which throw light on the process of determination. The limb rudiment can be regarded as a disc with anterior and posterior, dorsal and ventral and median and lateral, invisible axes. If a left limb bud is planted on the right side the proper way up and out (heteropleural dorso-dorsal antero-posterior), only the antero-posterior axis is reversed; as a result, it develops as a left limb. If on the other hand a left limb bud is planted on the right side the proper way out but upside down (hetero-
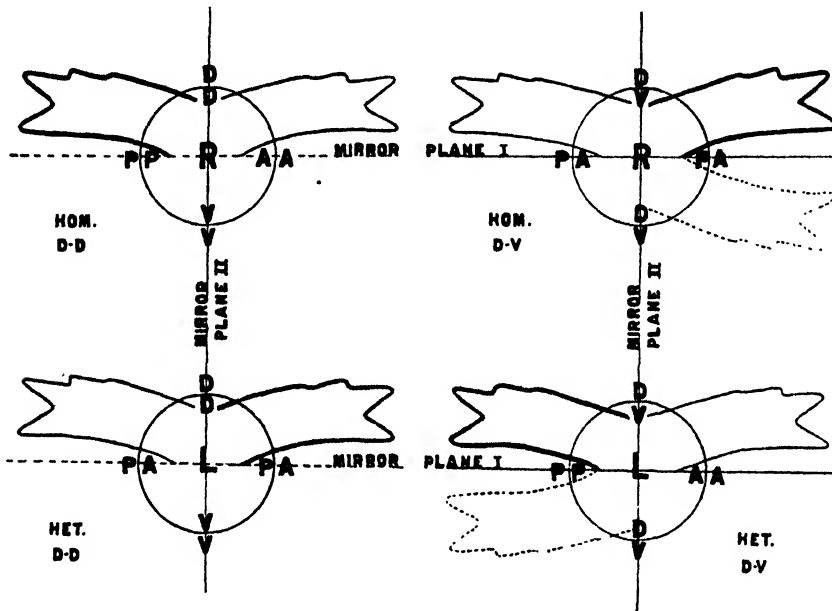
Fig. 7. Diagram illustrating experiments on the symmetry of limbs. The circles represent the limb buds as grafted on to the *right* side of the body. The letters R and L in the centre of the circles indicate the side of origin of the bud (right or left). The letters A, P, D, V, *inside* the circle indicate the antero-posterior and dorso-ventral axes of the grafted bud; these letters *outside* the circle refer to the same axes of the body of the organism. The limb which develops is shown with a thick outline. The position of a reduplicated limb (should one develop) is indicated by the fine outline; the dotted line refers to the form which the limb would have taken if the dorso-ventral axis of the bud had been fixed at the time of grafting. Only medio-medial combinations are shown. (From Harrison.)

pleural antero-anterior dorso-ventral), only the dorso-ventral axis is reversed. Such a bud develops into a right limb the proper way up. This means that the antero-posterior axis is not reversible; that it has been already fixed and that it determines the anterior and posterior margins of the limb. On the other hand the dorso-ventral axis is not fixed, and is reversed and regulated to conform to the same axis of the body of the animal into which it is grafted. Similarly with the medio-lateral axis, for a left mesoderm-rudiment transferred to the right side with proper antero-posterior and dorso-ventral orientation (heteropleural dorso-dorsal medio-

lateral) but median side out, develops into a right limb. By rotating a right bud 180° about the medio-lateral axis and replanting it *in situ* (homopleural antero-posterior dorso-ventral medio-medial), the remarkable result is achieved that a right bud produces a left limb on the right side of the body. These experiments were performed on *Amblystoma punctatum* at the tail-bud stage.

Brandt (1924) has obtained the same results in *Triton taeniatus* (in which the limbs appear much sooner) only at the neurula stage; at the tail-bud stage in *Triton* all the axes are fixed. Ruud (1926) has further corroborated these results on *Amblystoma tigrinum*. Here the limbs appear relatively later, and yet the antero-posterior axis is determined earlier. There is therefore no correlation between time of determination and time of appearance of the limb.

It is not the transposition of material from front to back which is responsible for these modifications, for if a rectangular graft is taken and halved vertically, and the two halves interchanged without reversing their orientation, a normal limb results (Harrison, 1925 *b*). The effects are therefore due solely to the orientation of the axes.

In all these cases it was very common for additional duplicated limbs to arise from the transplanted buds, and the duplicates are mirror-images of the main limbs,
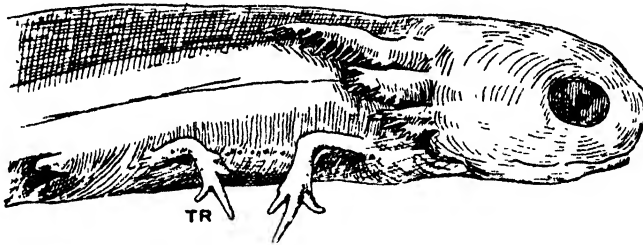
(Reproduced with permission from *Journ. Exper. Zool.* vol. **32**.)

Fig. 8. Axolotl in which an extra limb TR has developed from a grafted bud after homopleural dorso-ventral implantation. It is a "left" limb grown from a right bud on the right side. (From Harrison.)

and there may be two or more such duplicates on one limb; they may also arise at all levels on the limb. They obey Bateson's (1894) symmetry rule in that the long axes of the main limb and of its duplicate lie in the same plane, and each duplicate is with regard to the main limb, its image in a plane mirror bisecting the angle formed by the main limb and the duplicate at their point of junction, and placed at right angles to a line joining the corresponding structures of the main limb and the duplicate. The orientation and composition of these duplicates introduces the whole problem of asymmetry. Harrison (1921 *b*) is inclined to attribute asymmetry to an asymmetrical microstructure, perhaps of the molecular order.

Swett (1926) has further investigated the production of limb reduplications in *Amblystoma*. He finds that double limbs mirrored in the radial plane are much more numerous than those mirrored in the ulnar plane. Further, when there are two reduplicates, the radial one always develops first; when a reduplicate appears late it is always on the ulnar side. It is interesting to note in this connection that the

formation of digits takes place in a radio-ulnar direction, so that the radial border appears to be the more active of the two. In experiments in which a limb bud is
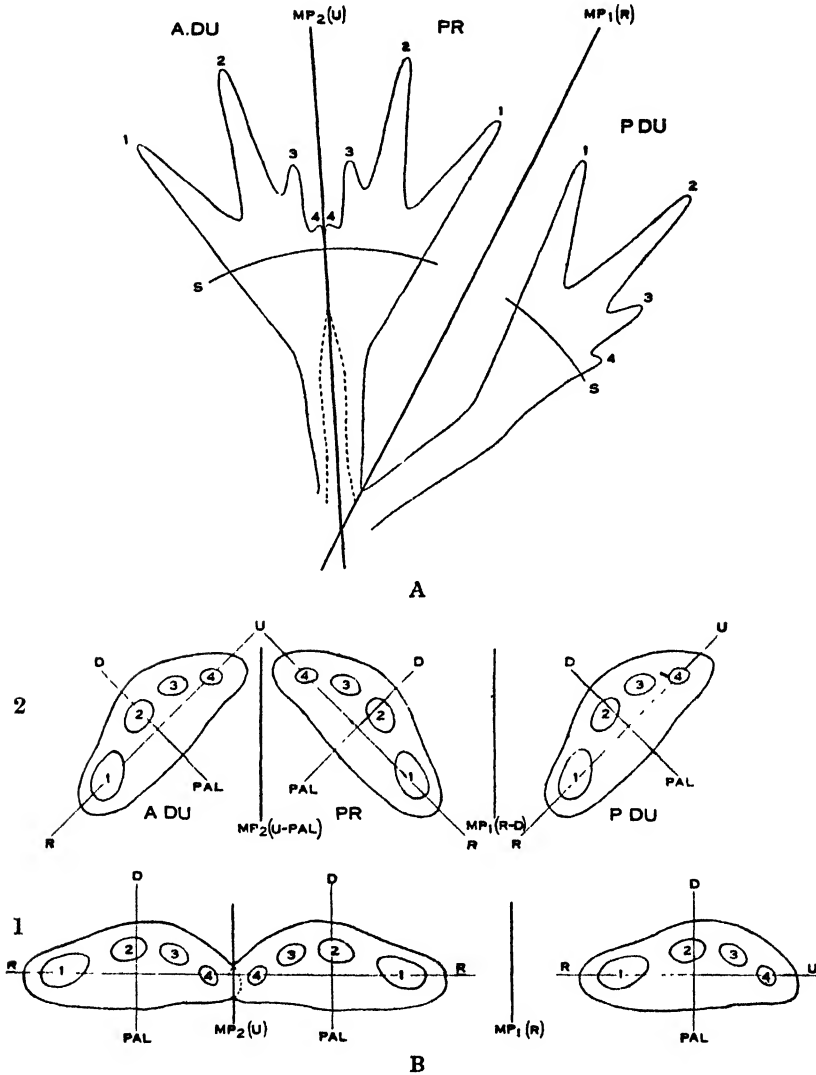
(Reproduced with permission from *Journ. Exper. Zool.* vol. 32.)

Fig. 9. Diagrams illustrating the symmetry relations of reduplicated limbs, PR, primary limb; P.DU, posterior reduplicated limb; A.DU, anterior reduplicated limb; MP₁, radial mirror plane; MP₂, ulnar mirror plane; the figures 1, 2, 3, 4 refer to the digits.
A. Lateral view of the limb complex. B. Sectional view of the limb complex taken through S-S in A. In 1, the mirror planes are radial and ulnar (at right angles to the radio-ulnar plane). In 2, the mirror planes are radio-dorsal and ulno-palmar (diagonal to the radio-ulnar plane). (From Harrison.)

split into two by grafting in a strip of indifferent tissue, each portion may form a normal limb. These pairs of limbs will be situated anteriorly and posteriorly or

dorsally and ventrally according to the direction of the split which divides the limb bud. The remarkable thing is that the less normally situated of such a pair of limbs (each of which is of the correct asymmetry for its side) always forms a mirror-image reduplication on the side nearest the other limb. The latter in some way influences the former.

Nicholas (1924 *a*) grafted limb buds of *Amblystoma* into the mid-dorsal and mid-ventral line. The result was that two limbs developed from the bud, and if the antero-posterior axis was correct with regard to the body of the animal, these limbs had the proper asymmetry for the side on which they were; if the antero-posterior axis was reversed, there was a right limb on the left side, and a left one on the right. It may be noticed that these cases are similar to reduplications as regards their symmetry relations.

Fig. 10. Longitudinal section of a femur developed from the proximal portion of a hindlimb bud of a chick incubated for 4 days. The bud was grafted on to the chorio-allantoic membrane of a 7-day chick and grown there for 5 days. Note the self-differentiation. (From Murray and Huxley.)

The important thing to note is that it is not laterality which is determined at this stage, but only the antero-posterior axis, *i.e.* the limb is determined in two but not in all three dimensions. The third dimension which makes the limb either right or left is determined by the dorso-ventral axis of the animal, regardless of the fact that by so doing the animal may develop a limb with the wrong asymmetry for the side.

The self-differentiation of limb buds is further well shown by Braus' (1905) experiments on *Bombinator*, in which he grafted the fore-limb rudiment (when it was still under the skin) on to the head, and into the place of an excised hind-limb rudiment. Wherever it was it developed into an arm, recognisable by the 4-fingered hand. Peebles (1910) extirpated the limb rudiment of the 4-day chick and found that no limb arose from any other cells. Strangeways and Fell (1926 *a*) took limb buds from the 81-hour chick, and grafted them under the skin of 11-day chicks. They differentiated into cartilage, bone, epidermis and fibrous tissue, but showed little relation as regards morphology to normal structure. They also cultivated the limb buds *in vitro*, and when grown in a rather solid medium they differentiated into cartilage, fibrous tissue and epidermis, bearing strong resemblances to a normal structure. No muscles were developed in either graft or culture.

Peebles (1910), using the 4-day chick, grafted the tip of the arm bud on to the

stump of the leg, and the tip of the leg on to the stump of the arm. It appeared that the grafts developed in conformity with their new situation, but the results are not clear. This means that regulation is still possible at this stage, and this result has been confirmed for regeneration-buds by Milojewic (1924).

The most definite results with regard to the determination of parts of the limb have been obtained by the method of grafting on to the chorio-allantoic membrane. Murray and Huxley (1925 b) took the basal part of the left posterior limb bud of the 4-day chick and cultivated it for 5 days as a graft. It differentiated into a perfect little left femur, with head and trochanter of cartilage with perichondrial bone. This experiment shows that a part of the limb bud is no longer capable of forming a whole at this stage. Its various regions are determined and are only capable of a very limited amount of regulation. This question has been further investigated by Murray (1926). The basal half of a 3-day limb bud differentiated into the proximal portion of a femur, while the apical half produced the distal portions of the femur, patella, tibia, fibula and foot. The following examples may be given of his experiments on the 4-day limb bud:

Basal half: complete femur; apical half: incomplete tibia, fibula, foot.

Basal half: proximal portion of humerus; apical half: distal portion of humerus, ulna, radius and hand.

Basal half: femur, proximal portion of tibia and fibula; apical half: complete (?) tibia, fibula and foot.

(If the tibia and fibula really are complete in the last case, then a certain amount of regulation must have taken place.) Basal quarter, perfect femur; second quarter, perfect tibia and fibula; apical quarter, perfect foot. Anterior and posterior halves likewise showed mosaic development. What structure differentiates in any given graft therefore depends on where the cuts were made in the limb bud: these parts must then be determined.

It is obvious therefore that after having been a partial equipotential harmonic system, the various regions become determined and the limb goes on to develop as a mosaic.

In several of these grafts, portions of the limb girdles appeared. Spurling (1923) found that in the 65-hour chick the separate parts of the pelvic girdle are already determined, and that removal of a portion results in a deficiency without regeneration. He also observed that the girdle rudiment at this stage would not regenerate a limb.

Braus (1909) transplanted the limb bud of *Bombinator*. In the place whence it came there developed only the distal ends of the suprascapula, coracoid and epicoracoid, and they were of normal size. The graft developed into a normal limb, and a perfect miniature shoulder girdle, one-third normal size. These transplanted portions of the girdle rudiment had therefore regulated. It is remarkable that the articulation between arm and girdle could not take place owing to size discrepancy, and yet each was perfectly formed. This is similar to Murray and Huxley's (1925 b) result with the chick femur, which had a well-formed head and trochanter. On the other hand, Meyer (1926) grafted the right arm bud of *Triton taeniatus*, at the

tail-bud stage, on to the left side of the head. The limb grew out normally, a shoulder girdle, deficient only in the suprascapula, was formed of one-third normal size, except for the glenoid cavity, which was of normal size, and fitted the head of the normal-size humerus. In this case, then, size regulation of the joint had taken place.

Harrison (1918) showed that in *Amblystoma* after extirpation of the limb-bud disc ($3\frac{1}{2}$ somites in diameter) portions of the suprascapula, coracoid and epicoracoid are left. These undergo hyperplasia and extend across the gap separating them. Detwiler (1918) showed that the separate parts of the girdle are already determined at the stage when the limb bud is a thickening of the body wall, and that the removal of a part was not followed by its restitution. The centre of the girdle rudiment is transplanted with an ordinary limb graft, and it develops into a girdle of one-third normal size. The dorsal half of the limb-bud zone is nearly free from the girdle rudiment. By transplanting portions of the bud, it is possible to obtain perfect limbs with deficient girdles.

At this stage, therefore, the girdle is not equipotential, while the limb is.

These results have been confirmed for *Triton taeniatus* by Brandt (1926).

Nicholas (1924 *b*), as result of experiments involving rotation of the limb bud, found that the symmetry of the girdle conformed to that of the limb, *i.e.* it might be wrong for its side, but it was not upside down. Harrison (1921 *b*) noticed that in some cases when the limb buds are rotated 180° and replanted on their own side, instead of developing with a reversed asymmetry, they underwent rotation at the shoulder joint, and eventually conformed to their side. Nicholas (1924 *b*) found that such limbs which had been rotated up to 235° righted themselves by reversed rotation. On the other hand, if they had been rotated through three right angles, they complete the circle by rotating the remaining right angle in the same direction. If the limb bud was only $1\frac{1}{2}$ somites in diameter, no girdle is formed, and this regulatory rotation does not take place. If the graft was 5 somites in diameter, a complete girdle was formed, and no regulatory rotation takes place (Nicholas, 1926). When in a graft 5 somites in diameter the $3\frac{1}{2}$-somite limb bud is separated from the rest, and both pieces are rotated independently, the limb undergoes postural regulation in regard to the peripheral piece, irrespective of its orientation to the organism as a whole (Nicholas, 1925).

Apparently the portions of the girdle (parts of suprascapula, coracoid and epicoracoid) whose rudiments are outside the $3\frac{1}{2}$-somite disc, but are included in a 5-somite transplant, act as determining factors with regard to their orientation on those portions of the girdle whose rudiments are included in the $3\frac{1}{2}$-somite disc. The girdle then determines the rotation of the limb. Obviously, then, in the $1\frac{1}{2}$-somite graft, no girdle being formed, no rotation can occur; in the 5-somite graft, the whole girdle is rotated and remains so; in the $3\frac{1}{2}$-somite graft, the central (transplanted) portions of the girdle unite with the normally orientated outer portions which had been left in the host, to form a complete girdle. This rotation of the girdle cannot take place as a whole, as shown by the fact that portions of pronephros transplanted with a graft do not move. So far then this rotation is a mystery.

That limbs can develop without nerve supply follows from Harrison's (1907) experiments already described in section 8 ("Aneurogenic" limb buds grafted after removal of the spinal cord of their embryo). Lebedinsky (1924) grafted a limb bud of *Pelobates* on to the ventral suface of the trunk in such a way that it hung by a long stalk. It developed normally, yet was demonstrably nerveless. Dauwart (1924) examined a reduplicated limb in *Pelobates* and found that it was also devoid of nerves.

Dürken (1916), on the other hand, from his experiments on *Rana fusca*, came to the conclusion that innervation was important for the development of grafted limb buds. The bud, consisting at the time of operation of mesenchyme and epidermis, was grafted into the place of the extirpated eye. It developed into either uninterpretable masses of cartilage and connective tissue, or into a more or less typical limb with musculature if it was innervated (by a branch of the trigeminal!). Other experiments by Groll (1924) appear to confirm this.

In other experiments, Dürken (1917, 1925 a) extirpated the leg rudiments of *Rana fusca* at a very early stage, and found that all three other limbs were deficient, as well as the nervous centres. Extirpation at a later stage did not produce this result on the limbs. The defects of the limbs were syndactyly, feebly developed toes, or long segments too long. Luther (1916) did not confirm these results, but they are partly supported by those of Hamburger (1925). He extirpated the right eye or the right optic lobe of the mid-brain in embryos of *Rana fusca*, and in 14 per cent. of cases the distal ends of the hind legs were deficient *in both legs*. The malformation took the form of suppression and reduction of digits 1, 2 and 5, the latest formed toes being the most affected. There can be no doubt that this effect is exerted through the nervous system (though non-specific, and probably due to general debility), and that innervation may therefore play a part in the later differentiation of the limb. It is interesting to compare these cases with those of hypotypic regeneration.

Mention must be made of three very curious sets of results which have been obtained in connection with limbs. Weber (1925) cauterised the hind-limb rudiments of one side in young tadpoles of *Rana fusca* with a fine platinum wire heated to redness. The results differed with the severity of the burn. If it was slight, the limb produced from the cauterised rudiment was one-quarter longer than normal. More severe burning resulted in a normal (or slightly smaller) limb, but the corresponding limb on the other side was a quarter longer than normal. When the burn was very severe, a very small limb or stump was formed and the corresponding other limb showed multiple reduplications. These experiments deserve repetition. Harrison (1924) made heteroplastic grafts between *Amblystoma tigrinum* and *A. punctatum*. The former species is the larger of the two, but limb buds appear first in the latter (in the embryonic period) and are well developed by the time when those of the former have made their first appearance. *A. punctatum* limb bud on *A. tigrinum* host gives rise to a limb which is not very remarkable, but a *tigrinum* limb bud on *A. punctatum* produces a limb which is at least twice the absolute size of the largest limb normally produced in either species.

The other puzzle arises out of Balinsky's (1925, 1926) grafting of an otic vesicle into the side of the trunk of *Triton cristatus*, at the late tail-bud stage. In certain cases a limb developed from the site of implantation. The possibility of a limb bud having been grafted with the ear is excluded. It is worth noticing that such a limb was nerveless. It is known that the otic vesicle has the power of inducing the formation of cartilage round itself, and perhaps the quality of such cartilage is determined by the region or "field" (Weiss, 1925) into which the otic vesicle is grafted.

Leaving the pentadactyl limb and turning to the fins of fish, some interesting and important results have been obtained by Braus (1906 *a*) in experiments on the Selachians *Scyllium* and *Pristiurus*. The first appearance of the fin is in the form of a fold of skin into which the muscle buds wander. Later on the cartilaginous radials appear, parallel to the muscles. Braus' first object was to see whether the formation of the cartilages was dependent on the muscles. To this end he cut a slit along the base of the fin parallel to the side of the body, and thereby prevented the muscle buds from entering. Nevertheless the cartilaginous radials developed. The latter normally appear first at the centre of the fin, and develop in succession, forward and backward. In order to find out whether this was a causal sequence or merely a temporal one, Braus made a cut in the fin perpendicular to the axis of the body. This did not prevent the entry of the muscle buds, and the formation of radials proceeded regularly up to the slit but no further. Beyond it there was merely an undivided plate of cartilage instead of separate radials. There is therefore good evidence that an impulse for the differentiations of cartilaginous radials travels, in some form or other, over the fin.

14. THE SKULL.

The results of experimental work relating to the skull are not numerous, though of considerable interest. Stone (1922) removed the neural crest from *Amblystoma* embryos at the tail-bud stage, and obtained deficiencies in the visceral skeleton corresponding to the region which had been removed. More recently (Stone, 1926) he has shown that removal of the neural crest and "mesectoderm" in the region of the trigeminal nerve results in great deficiencies in the palato-quadrate, Meckel's cartilage and the trabecula in front of the optic foramen, on the side operated upon. These remarkable results are difficult to interpret, as it is possible that the tissues removed may not themselves be the future constituents of the cartilages in question, but may influence their formation in some way. At the same time, by transplanting portions of the neural crest to the trunk, Stone obtained differentiation of cartilage. However, it is very interesting to see in these experiments support for the old suspicion that the trabeculae may be of visceral origin, and similar in nature to the jaws and arches.

At later stages these regions of the skull appear to be self-differentiating. Schaper (1898) removed the dorsal portions of the brain with the eyes and ears of newly hatched tadpoles of *Rana esculenta*. The wound healed and in the course

of a week the tadpoles had grown 2 mm. and it was found that the pterygo-palatine and the gill arches had differentiated properly.

Steinitz (1906), after removing the eye of a frog embryo, observed that the skull was malformed, but that the optic foramen was present though no optic nerve pierced it. It was however smaller than normal.

Burr (1916 a) removed the rudiment of the nose from embryos of *Amblystoma* 5 to 6 mm. long. Normally the nasal capsule is a cast of the nasal sac, but in these cases the capsule was completely collapsed. The cartilages were there, having self-differentiated from the mesenchyme, but they are dependent for their conformation on the nasal sac.

The cartilage of the auditory capsule has been proved to be dependent on the presence of the otic sac. Filatow (1916) found that removal of the otic sac from *Bufo* resulted in absence of the cartilaginous capsule. Reagan (1917) extirpated the otic sac from chick embryos; the capsule and the stapedial plate did not develop, the columella auris did. When displaced into strange mesenchyme, the otic sac induced the formation of a capsule. This result has been confirmed by Luther (1925) in *Rana esculenta*. The auditory capsule, operculum and pars interna plectri were shown to be dependent on the otic sac, the annulus tympanicus, pars media and pars externa plectri independent. Eisinger and Sternberg (1924) showed that even a small remnant of the vesicle is sufficient to induce the formation of cartilage.

Luther (1925) transplanted the otic sac to a region between the eye and the ear in *Rana esculenta* and obtained differentiation of cartilage: on the other hand if the graft was made into the trunk region, no capsule was formed. Filatow (1916) transplanted the otic sac from younger to older embryos of *Rana fusca*, and observed that the cartilage was formed from the tissues of the older host. Similarly Lewis (1906) grafted the otic sac of *Rana palustris* into *Amblystoma* and observed that the cartilage induced was formed from *Amblystoma* material. Stone (1926) however maintains that when an otic vesicle is transplanted in *Amblystoma*, a cartilaginous capsule only forms if mesectoderm is transplanted with it. This is improbable in view of Filatow's and Lewis' results.

Balinsky (1925, 1926) grafted the otic vesicle of *Triton cristatus* into the trunk at the late tail-bud stage, and in some cases obtained a limb. This result makes one wonder whether the cartilage which developed when Sternberg (1924) grafted the otic sac of *Rana fusca* to the ventral gill region might not be an attempt to form a visceral arch. It is perhaps worth mentioning that when Meyer (1926) grafted the limb bud of *Triton* on to the head, the auditory capsule was markedly smaller on the side of the graft. The relation of the palato-quadrates to the balancer in Urodeles is described in the next section.

15. THE GILLS, THE OPERCULUM AND THE BALANCER.

Harrison (1921 a) working on *Amblystoma* rotated the presumptive gill ectoderm at an early stage and obtained normal gills. At a later stage, however, rotation was followed by self-differentiation. The determining factors must lie in the deeper

tissues because transplantation of ectoderm from the head, heart and pronephric regions over the presumptive gill region gave rise to normal gills. On the other hand, trunk ectoderm could not be induced to form gills. On the whole the closer the seat of the origin of the graft to the normal gill region, the more perfectly does it develop into gills. At the later stage, when rotation through 180° leads to growth in the rotated position, the underlying tissue cannot be a well-defined mosaic, because two rudiments grafted together regulate to form normal gills provided that their antero-posterior orientation is correct.

These results are thoroughly supported by the experiments of Ekman (1913, 1922) on *Rana fusca, esculenta* and *Bombinator*. He found that if at the stage when the neural folds are just visible, the gill region was rotated 180°, normal gills were produced. If this experiment were repeated at a time between the neural plate and the gill plate stages, the gills developed reversed, and the operculum grew forwards instead of backwards. Other ectoderm in *Bombinator* grafted over the gill regions will give rise to gills provided that it comes from the head, heart or pronephric region, but not from the trunk. In *Rana fusca* and *esculenta*, however, any ectoderm can be made to form gills. After the first origin the circulation plays an important part in gill development, which is impeded on removal of the heart. Stöhr (1925) found that in *Bombinator*, stoppage of the afferent branchial circulation on one side resulted in under-development of the gills on that side.

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Fig. 11. Larva of *Bombinator*, 3 days after the ectoderm of the gill region on the left side had been rotated through 180°. * limit between the rotated and the normal ectoderm; *Ex*, position of the fore-limb; *Op'*, opercular fold; *K'*, gills. Note self-differentiation in the rotated position. (From Braus after Ekman.)

It may be remembered that Spemann (1921) showed that in *Triton* at the middle gastrula stage, presumptive nerve tube material of *taeniatus* grafted on to the gill region of *cristatus* proceeded to differentiate into gills, only it retained the *taeniatus* character of (precocious) development.

In Anurans when the arm develops it is covered over by the operculum, and in order to emerge the right arm perforates the operculum with its elbow. Braus (1906 *b*) showed that in *Bombinator* extirpation of the limbs was still followed by a perforation of the operculum which was smaller than normal. Ekman (1922) obtained the same result in *Rana arvalis*, Helff (1926) in *Rana pipiens* and *sylvatica*, and Braus (1920) in *Rana esculenta*, the perforation here being of normal size. These results were held (though as will be seen erroneously) to prove that the perforation was self-differentiating. At the same time Banchi (1905) had grafted an extra limb bud under the operculum in *Bufo*, and it induced its own perforation. The perforation was therefore dependent-differentiating also!—another alleged case of double assurance.

Later experiments have however shown that the perforation does not occur independently, though there is still confusion between the results.

Weber (1923, 1924) extirpated the arm bud in *Bombinator* and found that the perforation in the operculum formed if the limb stump were covered with glandular epithelium, but not otherwise. Helff (1924) paid attention to the fact that normal perforation of the operculum is accompanied by autolysis. Now when Braus (1906 *b*) grafted arm buds under the skin of the head, or Helff (1926) grafted them under the skin of the back, or even inserted glass beads under the skin, perforations occurred but without autolysis. At the same time all skin is capable of autolysis, as was proved by grafting back and side skin to the opercular region. (Helff's experiments were on *Rana clamitans*, *pipiens* and *sylvatica*.) Obviously then some structure other than limbs but near them is responsible for autolysis, as opercular skin grafted on to the back does not undergo autolysis. This structure has been proved to be the atrophying gills, for by transplanting resorbing gills beneath opercular skin on the back, autolysis was obtained. It appears then that the developing limbs accelerate the perforation (and localise it) by pressure. These cases show very well how provisional the description "self-differentiation" is.

The balancer is an organ present in some newts at the side of the head, and in the form of a long ectodermal tube with a mesodermal core. Bell (1907 *b*) found that in 3-mm. embryos of *Diemyctylus*, the ectoderm can differentiate into a balancer when transplanted without mesoderm. The determination therefore lies in the ectoderm, which induces the formation of a mesodermal core. It will only be regenerated if injuries are inflicted on its rudiment before it begins to grow out, *i.e.* early enough.

Harrison (1925 *a*) has confirmed these results in *Amblystoma punctatum*. At the neural fold stage its rudiment can be transplanted elsewhere on to the head, and it will develop by self-differentiation. *Amblystoma tigrinum* does not possess a balancer, but when a rudiment from *A. punctatum* is grafted on to it, it develops. Other epidermis grafted over the balancer region will not produce it. The degree of determination (for a long time invisible) becomes more and more pronounced with time; from young embryos the rudiment will only develop if grafted on to the head, from older ones the rudiment (still invisibly determined) will differentiate even if grafted on to the trunk. In grafts between embryos of different stages the balancer retains the "age" of its own original embryo.

The nerve supply is normally a twig of the mandibular branch of the trigeminal; and it can be innervated by this nerve in *Amblystoma tigrinum*, or by any other cranial or spinal nerve, and even by the trigeminal of *Rana sylvatica* when grafted on to this host.

At a late stage of development the balancer drops off, its basal membrane at its base becomes then embedded in the mesenchyme, and a cartilaginous extension of the palato-quadrate runs to meet it. Absence of the balancer entails absence of this process, as normally in *Amblystoma tigrinum*, in which however it may develop when a balancer is grafted on to it.

16. THE GUT, THE HEART, THE BLOOD, THE LIVER AND PANCREAS.

One of the results of Spemann's (1918) experiment on *Triton taeniatus* in which at the gastrula stage a piece of presumptive nerve-tube tissue together with the underlying gut roof was rotated through 180° and replanted, was that the embryo, which was perfect in all other respects, showed situs inversus viscerum et cordis (*i.e.* the stomach is on the right, the liver on the left, and the heart is twisted in the direction opposite that in the normal). Pressler (1906) obtained similar results in *Bombinator*, and Meyer (1913) in *Bufo vulgaris* and *variabilis*. Since the ventral regions of the gut had not been touched by the operations, the fact that not only the gut but also the heart was inverted as regards their asymmetry, shows that the latter is governed by some factor at this stage situated in the gut roof.

As a result of the ligaturing of *Triton* embryos in the gastrula stage leading to duplicitas anterior, Spemann and Falkenberg (1919) found that in 10 out of 12 cases, the right member of a pair was inverted, the left one normal. When the ligature was performed at an early stage and carried right through so as to give separate embryos from each lateral half of the gastrula, Ruud and Spemann (1923) found that the embryos developed from the left halves were normal, but half the number of those developed from the right half-gastrulae showed situs inversus. Wilhelmi (1921) suggested that this was due to the absence of a factor situated on the left side and whose function it was to determine the normal asymmetry. The left halves naturally possess this factor and are normal; the right ones lack it, and in these chance decides which way their asymmetry shall lie. She also extirpated regions from the left side and obtained situs inversus. This factor would also be supposed to function in the cases of the rotated gut roof, which in Spemann's (1918) experiments was a median piece. But when Meyer (1913) removed this piece of gut roof altogether, the hypothetical factor was absent, yet no situs inversus occurred. In the absence of such a factor it would be expected that half such animals would show situs inversus. The essential difference between Meyer's and Wilhelmi's experiments was that in Meyer's the deficiency of tissue was equal on both sides, in Wilhelmi's the deficiency was restricted to the left side. Warynsky and Fol (1884) obtained situs inversus in chicks by overheating on the left side. The result is that the right side is favoured.

Spemann's analysis of the question shows that the asymmetry of the viscera and heart may be due to: (i) an intimate asymmetrical microstructure, or (ii) a deficiency of material on one side. Since normally situs inversus is rare, and since artificial determination of the plane of symmetry (see Born, 1885; Jenkinson, 1909) does not lead to situs inversus, the asymmetry of the viscera must be determined at the time when the plane of bilateral symmetry is determined. Further, it is inconceivable that an external factor at this stage should always influence the egg in the direction of the same asymmetry; it is necessary to conclude that this determination lies with an intimate asymmetrical microstructure. This may refer to the sperm or to the egg structure itself. Reversal of this structure may cause

situs inversus. But Ruud and Spemann's and Wilhelmi's experimentally produced cases of situs inversus *need not* be due to reversal of the asymmetry of the micro-structure. The hypothesis that lack of material on one side may invert the viscera covers all the facts; in the left gastrula halves the lack is on the right which accentuates the normal asymmetry; in the right halves the lack is on the left, and this may reverse the normal asymmetry. Similarly in Wilhelmi's experiments, extirpation on the left side causes lack of material on that side. Further, the "lacking" side often has smaller eyes, limbs and body muscles. On the other hand, the results of rotating the gut roof seem to require the assumption of a localised factor for their explanation.

Mangold (1921) has attacked this problem very subtly. He found that normally about 2 per cent. of individuals of *Triton taeniatus* have situs inversus. It is known that 50 per cent. of right half-gastrulae have situs inversus. If this is due to reversal of the asymmetry of an intimate microstructure, then if the two blastomeres of the 2-cell stage be separated, 50 per cent. of the individuals developed from the right-hand blastomeres should be inverted. As a matter of fact only 3 per cent. are, a proportion very similar to the normal. These cases are therefore probably not due to reversal of an intimate microstructure. On the other hand, it is possible that the microstructure gets stronger in its effects as development proceeds, in which case there would be no reason to expect situs inversus from right halves at early stages. However, there is no reason why the microstructure factor should not coexist with the lack-of-material factor. This must indeed be so, for taking these cases of half-gastrulae and calling the microstructure factor A, the other B, then:

(i) That the asymmetry is normally constant can only be explained by A.

(ii) That only the right half-gastrulae show situs inversus can be explained by either A or B.

(iii) That not all the right half-gastrulae are inverse is only explicable by B.

Further support for the view that asymmetry and its reversal may be caused by either A or B is obtained from a consideration of Swett's (1921) observations on double trout monsters. Naturally only those monsters which are separate at least as far back as the hind end of the stomach can have two stomachs and show situs inversus at all. The important point is that those twins which are joined together only by the hinder region of the trunk (behind the abdominal cavity), or by the tail only, or which are quite separate, very rarely show situs inversus; whereas those which join together behind the stomach and in front of the end of the abdominal cavity frequently do in the right hand member. Some factor such as diminished material or diminished protoplasmic activity is therefore at work in the latter cases, where the asymmetrical regions in question are in close contact, to overcome the normal superior development of the left side, which must ultimately be due to an intimate microstructure. When these regions are quite separate, this disturbing factor is absent and the effects of the microstructure factor continue unmolested. In the beginning, probably all that factor A does is to confer a higher rate of activity and growth on the left side of the organism; and if as seems very probable this "dominance" of the left side increases during development, the time factor must be taken into consideration.

When the heart is inverted, the gut is so too in all cases, but the converse is not true. This shows that the asymmetry of the heart is dependent on that of the gut and suggests that it is not due to an asymmetrical microstructure, since it would be difficult to imagine it normal in one and reverse in the other.

Turning now to the determination of the heart, Stöhr (1925) proved that in *Bombinator* at the neural plate stage the heart rudiment could be rotated through 180° and still give a normal heart, while this is no longer possible at the tail-bud stage. Ekman (1921) showed that when the whole heart rudiment was removed at the neural plate stage, a heart was formed from neighbouring tissues, but not at a later stage. If at the neural fold stage a lateral half of the heart rudiment be removed, the remaining half gives rise to a normal heart, though in one case out of eight the right half gave an inverse heart (Ekman, 1925). Stöhr (1925) halved the rudiment at the neural plate stage, left one half in its embryo and grafted the other into another embryo from which the whole rudiment had been removed. The result was a normal heart in both embryos. Ekman (1925) grafted a piece of the lateral pharynx region into the heart rudiment in an early neurula, and observed (by means of *intra vitam* staining) that it took part in the formation of the heart. Power to be included in the heart decreases with distance from it. The heart rudiment can be augmented by planting in part of another; if this is done at the neural plate stage the whole will regulate to form a normal-sized heart, whereas at the tail-bud stage the heart so formed is abnormal.

After implantation of a piece of heart rudiment rotated through 180° into a slit in a heart rudiment at the neural plate stage, regulation does not take place and two hearts are formed, the right inverse. Ekman (1924) has obtained two and even three hearts in an organism by making longitudinal slits and preventing the cut edges from rejoining one another. At the neural plate stage, then, the heart rudiment is a harmonic equipotential system.

Stöhr (1924 *b*) showed the self-differentiating capacity of the rudiment by transplanting it to various positions in other individuals. The heart so formed enters into the circulation and pulsates independently of the host's own heart. It is remarkable that when grafted into abnormal positions the heart may grow to twice its normal size. The heart will also develop even if deprived of its blood supply, only it is then smaller than normal.

Results very similar to those just described in Anura were obtained in *Amblystoma punctatum* by Copenhaver (1926) who showed that any part as large as a half of the heart rudiment can form a complete heart, one rudiment can under experimental circumstances produce two hearts, and that two rudiments can regulate to form one heart, at the early tail-bud stage. It is therefore a harmonic equipotential system, but even at the earliest tail-bud stage the rudiment is already determined as regards the antero-posterior axis, as experiments of rotation show.

It will be remembered that in Murray and Huxley's (1925 *a*) grafted anterior third of the 24-hour chick blastoderm, the various regions of the heart must have been determined. In their specimen the bulbus and ventricle were present, but not the auricle or sinus.

Remarkable results have been obtained by culturing heart rudiments *in vitro.*
Ekman (1921) found that explants from the neural plate stage of *Bombinator* and
Rana esculenta differentiated into sinus, auricle, ventricle and bulbus, and pulsated
about 35 times a minute. Stöhr (1924 *a*) observed however that such hearts do not
have the proper torsion.

It is customary to regard the blood as an "organ," yet it is interesting to find
that in development in the frog it has a specific, definite and localised rudiment.
Frederici (1926) extirpated the median ventral blood island from embryos of *Rana
fusca* at the early tail-bud stage. If the extirpation was complete, the embryo had
no erythrocytes, and in cases of partial extirpation, the amount of erythrocytes
present was proportional to the amount of the rudiment which was left.

Holtfreter (1925) found that the rudiments of the liver and pancreas were already
determined at the late gastrula stage. When implanted in the yolk mass of another
embryo in the tail-bud stage they develop by self-differentiation. The tubules of
the liver develop if blood is present. It is remarkable that the rudiment of the gall
bladder is not localised at this stage, for it develops from grafts of the pars hepatica
of the liver as well as from those of the pars cystica.

The development of the rudiments of the viscera of the chick has been described
in section 7.

Adams (1924) has observed that in *Amblystoma* the mouth does not open unless
there is contact between the ectoderm and endoderm of the oral plate. It may be
recalled that Ekman (1923) showed that in *Rana fusca* the stimulus for the formation
of the choanae came from the nose. Cotronei (1921 *b*) obtained malformations of
the mouth in *Rana* and *Bufo*, as a result of treating the embryos with LiCl.

17. DEPENDENT DIFFERENTIATION AND SELF-DIFFERENTIATION: AXIAL GRADIENTS.

One of the most interesting questions arising out of consideration of the evidence
presented in the foregoing pages, refers to the relation between the dependent and
independent methods of differentiation of an organ. This question has been specially
treated by Spemann (1907 *b*), Becher (1912), Braus (1914), Brachet (1914) and
Dürken (1919), to mention only a few.

In many organs (*e.g.* lens of *Amblystoma*, gills of *Amblystoma*, *Rana* and *Bom-
binator*, nose, heart) it is plain that at first they are dependent on something else
for their determination, and that later they acquire a degree of independence which
increases with age. This means that the processes of chemo-differentiation take time,
and, according to the period when the organ is tested, it shows dependent or self-
differentiation.

There is no need to have recourse to the inheritance of acquired characters, or
rather transmission of self-induced modifications, in order to explain the case of
the opercular perforation. It is unnecessary and erroneous to speak of the cells of
the operculum having become so accustomed to perforate during the course of
countless generations that they can do so in the absence of the limb, when it is
known that perforation is no induced self-differentiating phenomenon, but is simply

dependent on the presence of the resorbing gills. Enough is known about the lens to suspect that it also at the start is dependent, even in *Rana esculenta* where its independence has already been achieved at the open neural fold stage. There is therefore no need to imagine either that the lens' self-differentiation is an induced and transmitted somatic modification, or that the separate determinations of eye and lens owe their interadaptiveness to a miraculous chance. On the contrary, it is becoming clearer all the time that correlations of this kind are due to chains of events occurring in *Ontogeny*, not in Phylogeny, and there is no basis for "double assurance" of the type discussed by Braus (1914) and Brachet (1914) among others.

In the final analysis nothing is self-differentiating, not even the organism itself. Child (1924), in a masterly sequence of reasoning, has shown that the origins of determinations, the most fundamental of which are polarity and symmetry, cannot have their sole explanation in internal factors. Since nuclear division is known not to be unequal, the complete idioplasm is present in every cell, and countless experiments have proved that the tissues of one region can have the potency to give rise to those of another. A special internal factor which would determine that such and such a region should become head and such another right side, falls under the same objection that it too would be distributed all over the organism.

As a matter of fact it is known that these early determinations are due to the environment; the apico-basal axis to the orientation of blood vessels in the ovary, the plane of bilateral symmetry to the entrance of the sperm. Only in the case of the normal visceral asymmetry does it appear to be necessary to appeal to an intimate microstructure, but if the latter resides in the sperm, then it too is external to the egg.

Given polarity and symmetry the only hypothesis which appears tenable is that of a Gradient System or "field" (Weiss, 1925) both for the origin of this polarity and symmetry, and for the place of determination of the rudiments of other organs. The most important feature of the theory is that qualitative differentiations are controlled by quantitative differences of rate of activity of protoplasm reacting on specific genes. This rate varies along a gradient, and at different "levels" on the gradient certain determinations take place. Before the frog's egg is fertilised, it is ready to form with its ventral side in *any direction*. This is completely unintelligible if the rudiments of the organism are internally self-differentiating. The very determining of the rudiments must bear a relation to the stimulus which fixes the plane of bilateral symmetry. As mentioned before, the first rudiment to be irrevocably determined is the organiser.

It is no part of the purpose of this review to enter in detail into the theory of Axial Gradients. It is, however, pertinent to enquire whether any of the evidence dealt with supports it. The following points are of interest in this connection:

(i) Huxley's demonstration (1926) that a temperature gradient passed through a frog's egg, translated into terms of protoplasmic activity, caused a modification of cell size in the blastula, of the rate of growth of the animal pole cells in the gastrula, and of the relative head size of the tadpole.

(ii) Bellamy's (1919) demonstration of regions of high protoplasmic activity

at the animal pole and dorsal lip of the blastopore in the frog's egg by various methods. These results have been criticised by Cannon (1923) but confirmed by Bellamy and Child (1924). (See also Hyman, 1921, 1926, 1927.)

(iii) Geinitz' (1925 b) observation that secondary embryos induced in *Triton* by organiser implantation are properly orientated in the antero-posterior axis with regard to that same axis of the host.

(iv) Spemann and Mangold's (1924) and Bautzmann's (1926) observation that in these organiser grafts, corresponding organs of the primary and secondary embryos tend to be at the same level with regard to the animal pole.

(v) Harrison's (1904) discovery that the path of the lateral line in frogs during its backward growth is not locally predetermined, and yet bears a definite position-relation to the whole organism, on an antero-posterior gradient, at a certain dorso-ventral level.

(vi) Harrison's, Ekman's, Stöhr's and Copenhaver's discoveries that in the rudiments of the limb buds, the gills and the heart, the first determination is that of the antero-posterior axis.

There is therefore strong support for the view that the various rudiments become determined at certain levels in a gradient co-ordinate system of which the abscissa culminates at the organiser and the ordinate at the animal pole. Gradient-determination is followed by progressive and soon irreversible chemo-differentiation *in situ*, as is proved by the increasing degree of independence of rudiments with age. In other words, this period in a rudiment represents the transition from the dependent to the independent differentiating condition.

In this manner it may be imagined that the rudiments are localised: gills, balancer, nose, ear, placodes, hypophysis, lens, limbs, liver, pancreas, heart and perhaps the nerve tube. At the same time it is quite clear that these segregates do not possess definite boundaries, but are to be regarded as centres of intensity of determination. This is shown very clearly by the fact that tissue capable of forming an organ does not form part of it in normal development (*e.g.* limb, lens).

Now that some of the activities and properties of the organiser are known, it may be asked whether the organiser functions as a self-sufficient, totipotent creator of differentiation, or whether it works on material which is already heterogeneous. The latter alternative is undoubtedly the correct answer to this question, but it must be understood that this heterogeneity is not of the nature of chemical differences of substance, but quantitative differences of rate of activity. The evidence for this may be briefly summarised as follows:

(i) Corresponding organs of primary and secondary embryos occur at the same parallel of latitude with regard to the original egg axis. This means that future potencies are already graded in respect of the primary apico-basal or antero-posterior axis. The same conclusion can be drawn from the fact that in normal development any given organ will always arise on a definite parallel of latitude whatever its meridian of longitude may be, the latter being determined by the point of entry of the sperm, or what comes to the same thing, the organiser.

(ii) Secondary embryos induced by an organiser implantation tend to lie along a meridian of longitude in respect of the original egg axis. This means that the

secondary medio-lateral gradients are at right angles to the egg axis, and determine the relations to the middle line of the organs which arise at any given parallel of latitude.

(iii) Raising or lowering the relative rate of protoplasmic activity at the top of the gradient results in an alteration of the proportional sizes of the head and the body. Thus in Huxley's (1927) experiments on the frog the head size was affected by heating or cooling the animal pole. Gowanloch's experiments (quoted by Child, 1924) on the fish *Macropodus viridi-auratus*, make use of the differential susceptibility of various regions to sub-lethal concentrations of toxic solutions (in this case atropine sulphate). Regions in which the protoplasmic rate is low are more adversely affected than those in which it is high, and which have a greater power of acclimatisation. The result of such treatment in these fish is that the heads are much larger than normal.

These cases fall strictly into line with those in which Child (1924, 1925) was able to alter the size of the head (or polyp) in *Planaria*, *Trochosphere* larvae and *Tubularia* by controlling the relative rate of protoplasmic activity at the top of the apico-basal axial gradient.

(iv) Once the plane of the bilateral symmetry has been fixed, the organs tend to form in relation to their appropriate gradients. This is obvious in the case of the lateral line, but the nerve tube is of greatest importance in this connection. In Ruud and Spemann's (1923) half-gastrulae when the cut coincides with the plane of bilateral symmetry, the cut edges approximate to one another; with the result that the presumptive nerve-tube material no longer lies in a straight line but is bent out of shape and deflected to one side. If the organiser paid no attention to gradients in the material on which it works, the nerve tube should arise straight in front of it. As a matter of fact, it follows the curve which the presumptive material has been forced to make. The organiser therefore works on tissues whose fates are in part the result of the gradient system, and this is why Goerttler (1925) was able to get a certain amount of differentiation of the nerve tube after removing the organiser, and Dürken (1925 *b* and 1926) was able to get pieces of *blastulae* of *Rana fusca* to differentiate. But it must be remembered that the organiser had already long before determined the medio-lateral gradients and the plane of bilateral symmetry. There is danger of grave confusion if it is not realised that the pre-organiser and organiser exercises two functions:

(i) Determination of the plane of bilateral symmetry and localisation of future potencies with reference to this plane and to the egg axis, *i.e.* giving longitude to the egg in addition to the latitude which it already has.

(ii) Invagination as gut roof and evoking the appropriate response from the overlying previously gradiented material. There must therefore be a progressive development of the functions of the organiser.

When an organiser is grafted into the flank of another embryo, both these functions are exercised, and it must be noted that in regard to the former there must be a certain amount of interference in respect of the host's own organiser of the region in which the organiser is planted.

This view of the conditions in the newt's egg lessens the differences between it

and the observed results in the frog's egg. There is however no need to be astonished at the fact that in the latter the gradienting determinative processes appear to act faster than in the former.

Again it may be emphasised that in the determination of regions in a gradient system, it is not the *absolute* rate of activity but the *relative* which is of importance. In other words there must be a "potential difference" of a certain order between regions of high and low rates, with, presumably "epistatic minima."

Coupled with the determination of a region to differentiate into a certain tissue is the loss of potency of other regions to differentiate into that same tissue. This is reflected in the loss of power to form an organ by tissues at increasing distances from the site of that organ. Especially well does this appear in the case of the power of head and trunk tissue to form lens, heart and gills in *Bombinator*.

The processes of chemo-differentiation proceed at different rates in different species. So may be explained the fact that in *Rana esculenta* the lens is already determined at the neural fold stage, while in *Rana fusca* it is not determined until much later. That which determines the lens in *Rana esculenta* must be situated in the rudiment of the eye, and it must persist until the eye touches the epidermis. So it can be understood how the eye of *esculenta* can induce lens formation from strange skin of *Bufo* and *Bombinator*, and how the lens normally arises in *Rana fusca*. Another example of the different speeds at which the gradienting processes work is to be found in the case of the determination of the antero-posterior axis of the limb bud in *Amblystoma punctatum*, *A. tigrinum* and *Triton taeniatus*.

By persistence of the original determining influence, the stage of dependent differentiation often overlaps that of self-differentiation. This it is which simulates "double assurance" and which explains such cases as that of the eye of *Rana esculenta* and its lens-inducing properties.

Owing to these developmental variations, there may be danger in attributing phylogenetic significance to ontogenetic details.

It is to be hoped that future work will not neglect the nature of the transitions from dependent to self-differentiation, and the parts played by physical and chemical processes. Child (1924) has made it probable that the very first differentiation of all is of a dynamic nature, based on transmission of excitation and resulting in "Axiation." When the axiate pattern has been set up, chemical differences may arise between different regions. Stress has already been laid on the difference between chemical (histological) and mechanical (morphological) differentiation. Chemo-differentiated tissue is an "organ-forming substance," such as Conklin (1924) has demonstrated in the ascidian *Styela* by centrifuging experiments. Duesberg (1926) has shown that some of these organ-forming substances are mitochondria. In the frog, however, Jenkinson (1914) showed that normal morphological differentiation could ensue even if the yolk, fat and protoplasm of the egg were quite considerably disarranged. Thus the brain could be normally formed although it contained much more than the normal quantity of fat. Too great a disturbance of course prevents development. Yolk and fat are therefore not specific organ-forming substances but raw materials.

It is obvious that one cannot speak of the "embryo-in-the-rough" until after the stage of chemo-differentiation has started. From that time on, development proceeds largely controlled by internal factors, but always limited by external temperature, humidity, oxygen, osmotic pressure, and later on, function.

But the egg only becomes the embryo-in-the-rough as a result of the impinging on it of two external stimuli. The first evokes the capacity of the egg to become radially symmetrical about any axis. The second brings out its capacity to become bilaterally symmetrical in one of an infinity of planes passing through that axis. Thereafter differentiation arises. At last the Preformation-Epigenesis question has been answered: "Heredity does not account for the individual, but merely for the potentialities some of which are realised in the individual." These potentialities are realised as a result of external stimuli (Epigenesis). Of Preformation in the old sense of spatial prearrangement of already differentiated materials it is of course impossible to speak. The only predetermination which exists concerns the potentialities just mentioned and ensures that if they are realised at all, the resulting organism shall belong to the same species as its parents.

LIST OF REFERENCES.

ADAMS, A E. (1924). *Journ. Exp. Zool.* 40, 311.
AGASSIZ, A. and DANCHAKOFF, V. (1922). *Anat. Rec.* 23, 7.
ANASTASI, O. (1913). *Arch. Ent. Mech.* 37, 222.
ATTERBURY, R. R. (1923). *Amer. Journ. Anat.* 31, 409.
BALDWIN, W. M. (1915). *Anat Rec.* 9, 365
—— (1919). *Biol. Bull.* 37, 294.
BALINSKY, B. I. (1925). *Arch. Ent. Mech.* 105, 718.
—— (1926) *Arch. Ent. Mech.* 107, 679.
BANCHI, A. (1905). *Arch. Ital. Anat. Embr.* 4, 671.
BATAILLON, E. (1904). *Arch. Ent. Mech.* 18, 1.
BATESON, W. (1894) *Materials for the study of Variation.* London.
BAUTZMANN, H. (1926). *Arch. Ent. Mech.* 107, 283.
BECHER, H. (1912). *Zool. Jahrb.* Sup. 15, 3, 501.
BELL, E. T. (1906). *Anat. Anz.* 29, 185.
—— (1907 *a*). *Arch. Ent. Mech.* 23, 457.
—— (1907 *b*). *Anat. Anz.* 31, 283.
BELLAMY, A. W. (1919). *Biol. Bull.* 37, 312.
—— (1921). *Biol. Bull.* 41, 351.
BELLAMY, A. W. and CHILD, C. M. (1924). *Proc. Roy. Soc.* B, 96, 132.
BORN, G. (1885). *Arch. Mikr. Anat.* 24, 475.
—— (1897). *Arch. Ent. Mech.* 4, 349, 517.
BRACHET, A. (1905). *Arch. de Biol.* 21, 103.
—— (1906). *Arch. Ent. Mech.* 22, 325.
—— (1912). *C. R. Acad. Sci.* 155, 1191.
—— (1913). *Arch. de Biol.* 28, 447.
—— (1914). *C. R. Soc. Biol.* 77, 557.
—— (1917). *L'œuf et les facteurs de l'ontogénèse.* Doin, Paris.
—— (1923). *Arch. de Biol.* 33, 343.
BRANDT, W. (1924). *Arch. Mikr. Anat. und Ent. Mech.* 103, 517.
—— (1926). *Ver. Anat. Ges.* 35, 36.
BRAUS, H. (1905). *Anat. Anz.* 26, 433.
—— (1906 *a*). *Morph. Jahrb.* 35, 340.
—— (1906 *b*). *Morph. Jahrb.* 35, 509.
—— (1909). *Morph. Jahrb.* 39, 155.
—— (1911). *Münchner Med. Woch.* 58 (2), 2420.

BRAUS, H. (1914). *Zeit. Morph. und Anthrop.* **18**, 65.
—— (1920). *Ver. Nat. Hist. Med. Verein.* Heidelberg, N.F. **14**, 215.
BURR, H. S. (1916 *a*). *Journ. Exp. Zool.* **20**, 27.
—— (1916 *b*). *Journ. Comp. Neur.* **26**, 203.
—— (1920). *Journ. Exp. Zool.* **30**, 159.
—— (1924). *Proc. Soc. Exp. Biol. and Med.* **21**, 473.
CANNON, H. G. (1923). *Proc. Roy. Soc.* B, **94**, 232.
CHAMBERS, R. (1922). *Anat. Rec.* **24**, 1.
CHILD, C. M. (1924). *Physiological foundations of Behavior.* Holt, New York.
—— (1925). *Anat. Rec.* **31**, 369.
COLE, W. H. (1922). *Journ. Exp. Zool.* **35**, 353.
CONKLIN, E. G. (1924). *General Cytology.* Chicago Univ. Press.
COPENHAVER, W. M. (1926). *Journ. Exp. Zool.* **43**, 321.
COTRONEI, G. (1921 *a*). *Ricerche di Morph.* **2**, 1.
—— (1921 *b*). *Riv. di Biol.* **3** (4), 1.
—— (1921 *c*). *Arch. Ital. de Biol.* **71**, 1.
DANCHAKOFF, V. (1916). *Amer. Journ. Anat.* **20**, 255.
—— (1922). *Anat. Rec.* **23**, 14.
—— (1924). *Zeit. Anat. und Entwick.* **74**, 401.
DAUWART, A. (1924). *Latvijas. Univers. Raksti',* Riga, **9**, 157.
DE BEER, G. R. (1926). *Introduction to Experimental Embryology.* Oxford Univ. Press.
DETWILER, S. R. (1917). *Anat. Rec.* **13**, 493.
—— (1918). *Journ. Exp. Zool.* **25**, 499.
—— (1920). *Journ. Exp. Zool.* **31**, 117.
—— (1922). *Journ. Exp. Zool.* **35**, 115.
—— (1923 *a*). *Journ. Exp. Zool.* **37**, 339.
—— (1923 *b*). *Journ. Exp. Zool.* **38**, 293.
—— (1924). *Proc. Nat. Acad. Sci.* **10**, 64.
—— (1925 *a*). *Journ. Exp. Zool.* **41**, 293.
—— (1925 *b*). *Journ. Exp. Zool.* **42**, 333.
—— (1926). *Journ. Exp. Zool.* **45**, 399.
DRIESCH, H. (1921). *Philosophie des Organischen.* Engelmann, Leipzig.
DUESBERG, J. (1926). *L'œuf et ses localisations germinales.* Presses Universitaires, Paris.
DÜRKEN, B. (1912). *Zeit. Wiss. Zool.* **99**, 189.
—— (1913). *Zeit. Wiss. Zool.* **105**, 192.
—— (1916). *Zeit. Wiss. Zool.* **115**, 58.
—— (1917). *Biol. Zentrbl.* **37**, 127.
—— (1919). *Einführung in die Experimentalzoologie.* Springer, Berlin.
—— (1925 *a*). *Biol. Zentrbl.* **45**, 541.
—— (1925 *b*). *Ver. Deut. Zool. Ges.* **30**, 84.
—— (1926). *Arch. Ent. Mech.* **107**, 727.
EISINGER, K. and STERNBERG, H. (1924). *Arch. Mikr. Anat. und Ent. Mech.* **100**, 542.
EKMAN, G. (1913). *Morph. Jahrb.* **47**, 419.
—— (1914 *a*). *Arch. Ent. Mech.* **39**, 328.
—— (1914 *b*). *Arch. Ent. Mech.* **40**, 121.
—— (1921). *Finska Vetensk. Soc. Forh.* **63**, Part 5, 1.
—— (1922). *Com. Biol. Soc. Scient. Fenn.* **1**, Part 3, 1.
—— (1923). *Com. Biol. Soc. Scient. Fenn.* **1**, Part 6, 1.
—— (1924). *Com. Biol. Soc. Scient. Fenn.* **1**, Part 9, 1.
—— (1925). *Arch. Ent. Mech.* **106**, 320.
FESSLER, F. (1920). *Arch. Ent. Mech.* **46**, 169.
FILATOW, D. (1916). *Rev. Zool. Russ.* **1**, 48.
—— (1924). *Arch. Mikr. Anat. und Ent. Mech.* **104**, 50.
—— (1925). *Arch. Ent. Mech.* **105**, 475.
—— (1926). *Arch. Ent. Mech.* **107**, 575.
FISCHEL, A. (1917). *Arch. Ent. Mech.* **42**, 1.
—— (1921). *Arch. Ent. Mech.* **49**, 83.
FREDERICI, E. (1926). *Arch. de Biol.* **36**, 465.
GEINITZ, B. (1925 *a*). *Arch. Ent. Mech.* **106**, 357.
—— (1925 *b*). *Zeit. Ind. Abst. und Vererb.* **37**, 117.
GIERSBERG, H. (1924). *Arch. Mikr. Anat. und Ent. Mech.* **103**, 368.
GOERTTLER, K. (1925). *Arch. Ent. Mech.* **106**, 503.
—— (1926). *Zeit. Anat. und Entwick.* **80**, 283.
GOODALE, H. D. (1911). *Amer. Journ. Anat.* **12**, 173.
GROLL, O. (1923). *Arch. Mikr. Anat. und Ent. Mech.* **100**, 385.

HAMBURGER, V. (1925). *Arch. Ent. Mech.* **105**, 149.
HARRISON, R. G. (1904). *Arch. Mikr. Anat.* **63**, 35.
—— (1907). *Journ. Exp. Zool.* **4**, 239.
—— (1910). *Journ. Exp. Zool.* **9**, 787.
—— (1912). *Anat. Rec.* **6**, 181.
—— (1918). *Journ. Exp. Zool.* **25**, 413.
—— (1919). *Proc. Soc. Exp. Biol. and Med.* **17**, 199.
—— (1921 *a*). *Biol. Bull.* **41**, 156.
—— (1921 *b*). *Journ. Exp. Zool.* **32**, 1.
—— (1924). *Proc. Nat. Acad. Sci.* **10**, 69.
—— (1925 *a*). *Journ. Exp. Zool.* **41**, 349.
—— (1925 *b*). *Arch. Ent. Mech.* **106**, 409.
HELFF, O. M. (1924). *Anat. Rec.* **29**, 102.
—— (1926). *Journ. Exp. Zool.* **45**, 1.
HERBST, C. (1901). *Formative Reize in der Tierischen Ontogenese.* Georgi, Leipzig.
HERLANT, M. (1911). *Arch. de Biol.* **26**, 103.
HERLITZKA, A. (1897). *Arch. Ent. Mech.* **4**, 624.
HERTWIG, G. (1913). *Arch. Mikr. Anat.* **81** (2), 87.
—— (1925). *Arch. Ent. Mech.* **105**, 294.
HERTWIG, O. (1893). *Arch. Mikr. Anat.* **42**, 662.
—— (1913). *Arch. Mikr. Anat.* **82** (2), 1
HERTWIG, P. (1913). *Arch. Mikr Anat.* **81** (2), 173.
—— (1916). *Arch. Mikr. Anat.* **87** (2), 63.
—— (1924). *Arch. Mikr. Anat und Ent. Mech.* **100**, 41.
HOADLEY, L. (1924). *Biol. Bull.* **46**, 281.
—— (1925 *a*). *Journ. Exp Zool.* **42**, 143.
—— (1925 *b*). *Journ. Exp. Zool.* **42**, 163
—— (1926 *a*) *Journ. Exp. Zool.* **43**, 151.
—— (1926 *b*). *Journ. Exp. Zool.* **43**, 179.
—— (1926 *c*). *Journ. Exp. Zool.* **43**, 197.
—— (1926 *d*). *Arch. de Biol.* **36**, 225.
HOLT, E (1921). *Anat. Rec.* **22**, 207.
HOLTFRETER, J. (1925). *Arch. Ent. Mech.* **105**, 330.
HUXLEY, J. S (1924). *Nature*, **113**, 273.
—— (1926). *Anat Rec.* **34**, 126.
HYMAN, L. H (1916). *Journ. Exp Zool.* **20**, 99
—— (1921). *Biol. Bull.* **40**, 32.
—— (1926). *Journ. Morph.* **42**, 111.
—— (1927). *Biol. Bull.* **52**, 1
HYMAN, L H. and BELLAMY, A. W. (1922) *Biol. Bull.* **43**, 313.
INGVAR, S. (1919). *Proc. Soc. Exp. Biol. and Med.* **17**, 198.
JENKINSON, J. W. (1906). *Arch. Ent. Mech.* **21**, 367.
—— (1909). *Biometrika*, **7**, 148
—— (1913). *Experimental Embryology.* Oxford Univ. Press.
—— (1914). *Quart. Journ Micr. Sci.* **60**, 61.
—— (1917). *Lectures on Experimental Embryology.* Oxford Univ. Press
KAAN, H W. (1926). *Journ. Exp. Zool.* **46**, 13.
KASTCHENKO, N. (1888). *Anat. Anz.* **3**, 445.
KING, H. D. (1905). *Arch. Ent. Mech.* **19**, 85
KOPSCH, F. (1896) *Ver. Anat. Ges.* **10**, 113.
LEBEDINSKY, N. G. (1923). *Anat. Anz.* **56**, 257
—— (1924). *Arch. Ent. Mech.* **102**, 101.
LE CRON, W. L. (1906). *Amer. Journ. Anat.* **6**, 245.
LEHMANN, F. E. (1926). *Arch. Ent. Mech.* **108**, 243.
LEVY, O. (1906). *Arch. Ent. Mech.* **20**, 335.
LEWIS, W. H. (1904). *Amer. Journ. Anat.* **3**, 505.
—— (1905). *Journ. Exp. Zool.* **2**, 431.
—— (1906). *Anat. Rec.* **1**, 141.
—— (1907 *a*). *Amer. Journ. Anat.* **6**, 473.
—— (1907 *b*). *Amer. Journ. Anat.* **7**, 137.
—— (1907 *c*). *Amer. Journ. Anat.* **6**, 461.
—— (1908). *Amer. Journ. Anat.* **7**, 259.
—— (1909). *Anat. Rec.* **3**, 175.
—— (1912). *Anat. Rec.* **6**, 1, 325.
LEWY, F. (1913). *Arch. Mikr. Anat.* **82** (2), 65.

LUTHER, A. (1916). *Finska Vetensk. Soc. Forh.* **58** (18), 1.
—— (1925). *Comm. Biol. Soc. Sci. Fenn.* **2**, 1.
MacWHORTER, J. E. and WHIPPLE, A. L. (1912). *Anat. Rec.* **6**, 121.
McCLENDON, J. F. (1910). *Amer. Journ. Anat.* **10**, 425.
—— (1912). *Amer. Journ. Phys.* **29**, 289.
MANGOLD, O. (1920). *Arch. Ent. Mech.* **47**, 249.
—— (1921). *Arch. Ent. Mech.* **48**, 505.
—— (1924). *Arch. Mikr. Anat. und Ent. Mech.* **100**, 198.
—— (1925). *Ver. Deut. Zool. Ges.* **30**, 50.
MARX, A. (1925). *Arch. Ent. Mech.* **105**, 19.
MAY, R. M. and DETWILER, S. R. (1926). *Journ. Exp. Zool.* **43**, 83.
MENCL, E. (1903). *Arch. Ent. Mech.* **16**, 328.
MEYER, R. (1913). *Arch. Ent. Mech.* **37**, 85.
MEYER, T. (1926). *Arch. Ent. Mech.* **108**, 388.
MILOJEWIC, B. D. (1924). *Arch. Mikr. Anat. und Ent. Mech.* **103**, 80.
MINOURA, T. (1921). *Journ. Exp. Zool.* **33**, 1.
MORGAN, T. H. (1895). *Anat. Anz.* **10**, 623.
—— (1905). *Arch. Ent. Mech.* **19**, 566.
MURPHY, J. B. (1916). *Journ. Exp. Med.* **24**, 1.
MURPHY, J. B. and ROUS, P. (1912). *Journ. Exp. Med.* **15**, 119.
MURRAY, P. D. F. (1926). *Proc. Linn. Soc. New South Wales*, **51**, 187.
MURRAY, P. D. F. and HUXLEY, J. S. (1925 a). *Brit. Journ. Exp Biol.* **3**, 9.
—— —— (1925 b). *Journ. Anat.* **59**, 379.
NEWMAN, H. H. (1917). *Biol. Bull.* **32**, 306.
—— (1923). *The Physiology of Twinning.* Chicago Univ. Press.
NICHOLAS, J. S. (1924 a). *Journ. Exp. Zool.* **39**, 27.
—— (1924 b). *Journ. Exp Zool.* **40**, 113.
—— (1925). *Anat. Rec.* **29**, 108.
—— (1926). *Anat. Rec.* **32**, 218.
OGAWA, C. (1921). *Journ. Exp. Zool.* **34**, 17.
—— (1922). *Bericht Jap. Med. Ges.* **6**.
—— (1926). *Fol. Anat. Japon.* **4**, 413.
PEEBLES, F. (1898). *Arch. Ent. Mech.* **7**, 405.
—— (1910). *Biol. Bull.* **20**, 14
PETERFI, T. (1924). *Mikrurgische Methodik.* Abderhalden's Handbuch der biologischen Arbeits-
    methoden. Urban und Schwarzenberg, Berlin.
PETERSEN, H. (1923). *Zeit. Ges. Anat. Ergeb.* **24**, 327.
—— (1924). *Zeit. Ges. Anat. Ergeb.* **25**, 623.
PRESSLER, K. (1906). *Arch. Ent. Mech.* **22**, 207.
PRZIBRAM, H. (1926). *Tierpfropfung.* Vieweg, Braunschweig.
RABL, C. (1917). *Arch. Mikr. Anat.* **90**, 261.
REAGAN, F. P. (1917). *Journ. Exp. Zool.* **23**, 85.
REAGAN, F. P., MacMORLAND, E. E., and MUDD, S. (1917). *Anat. Rec.* **12**, 265.
RIENHOFF, W. F. (1922). *Johns Hop. Hosp. Bull.* **33**, 392.
ROUX, W. (1895 a). *Gesammelte Abhandlungen*, **2**, 19.
—— (1895 b). *Gesammelte Abhandlungen*, **2**, 22.
—— (1903). *Anat. Anz.* **23**, 65, 113, 161.
RUUD, G. (1925). *Arch. Ent. Mech.* **105**, 209.
—— (1926). *Journ. Exp. Zool* **46**, 121.
RUUD, G. and SPEMANN, H. (1923) *Arch. Ent. Mech.* **52**, 95.
SCHAPER, A. (1898). *Arch. Ent. Mech.* **6**, 151.
SCHULTZE, O. (1894). *Arch. Ent. Mech.* **1**, 269.
SHORREY, M. L. (1909). *Journ. Exp. Zool.* **7**, 25.
SMITH, B. G. (1914). *Biol. Bull.* **26**, 245.
SMITH, P. E. (1920). *Amer. Anat. Mem.* **11**.
SPEMANN, H. (1901 a). *Arch. Ent. Mech.* **12**, 224.
—— (1901 b). *Ver. Anat. Ges.* **15**, 61.
—— (1902). *Arch. Ent. Mech.* **15**, 448.
—— (1903). *Arch. Ent. Mech.* **16**, 551.
—— (1904). *Zool. Jahrb.* (Abt. Zool. und Phys.), Sup. 7, p. 429.
—— (1905). *Zool. Anz.* **28**, 419.
—— (1906 a). *Ver. Ges. Deut. Nat. und Ärzte*, **78**, 189.
—— (1906 b). *Ver. Deut. Zool. Ges.* **16**, 195.
—— (1906 c). *Naturwissenschaften*, **21**, 200.
—— (1907 a). *Zool. Anz.* **31**, 379.

SPEMANN, H. (1907 b). *Ver. Deut. Zool. Ges.* **17**, 22.

—— (1908). *Ver. Deut. Zool. Ges.* **18**, 101.

,—— (1910). *Arch. Ent. Mech.* **30** (2), 437.

—— (1912 a). *Zool. Jahrb. (Abt. Zool. und Phys.)*, **32**, 1.

—— (1912 b). *Zool. Jahrb.* Sup. 15 (Festschr. für Spengel), **3**, 1.

—— (1914). *Ver. Deut. Zool. Ges.* **24**, 216.

—— (1916). *Sitzber. Ges. Nat. Freunde Berlin*, p. 306.

—— (1918). *Arch. Ent. Mech.* **43**, 448.

—— (1919). *Naturwissenschaften*, Heft **32**.

—— (1921 a). *Mikrochirurgische Operationstechnik.* Abderhalden's Handbuch der biologischen Arbeitsmethoden. Urban und Schwarzenberg, Berlin.

—— (1921 b). *Arch. Ent. Mech.* **48**, 533.

—— (1924). *Zeit. Ind. Abst. und Vererb* **33**, 272.

—— (1925). *Brit. Journ. Exp. Biol.* **2**, 493.

SPEMANN, H. and FALKENBERG, H. (1919). *Arch. Ent. Mech.* **45**, 371.

SPEMANN, H. and MANGOLD, H. (1924). *Arch. Mikr. Anat. und Ent. Mech.* **100**, 597.

SPURLING, R. G. (1923). *Anat. Rec.* **26**, 41.

STEINITZ, E. (1906). *Arch. Ent. Mech.* **20**, 537.

STERNBERG, H. (1924). *Arch. Mikr. Anat. und Ent. Mech.* **103**, 259.

STOCKARD, C. R. (1907). *Amer. Journ. Anat.* **6**, 511.

—— (1909). *Journ. Exp. Zool.* **6**, 285.

—— (1910 a). *Amer. Journ. Anat.* **10**, 369

—— (1910 b). *Amer. Journ Anat.* **10**, 393.

—— (1913). *Amer. Journ. Anat.* **15**, 253.

—— (1921). *Amer. Journ Anat.* **28**, 115.

STÖHR, P. jun. (1924 a). *Arch Ent. Mech.* **102**, 426.

—— (1924 b). *Arch. Ent. Mech.* **103**, 555.

—— (1925). *Arch. Ent Mech.* **106**, 409.

STONE, L. S. (1922). *Journ Exp. Zool.* **35**, 421.

—— (1924). *Journ. Comp New* **38**, 73

—— (1925). *Anat Rec.* **29**, 375

—— (1926). *Journ. Exp. Zool* **44**, 95.

STRANGEWAYS, T. S. P. and FELL, H. B. (1926 a). *Proc. Roy. Soc. B*, **99**, 340.

—— —— (1926 b). *Proc. Roy. Soc. B*, **100**, 273.

STREETER, G. L. (1906). *Journ. Exp. Zool.* **3**, 543.

—— (1907). *Journ. Exp. Zool.* **4**, 431

—— (1909). *Anat. Rec.* **3**, 199.

—— (1914). *Journ. Exp Zool.* **16**, 149.

SWETT, F. H. (1921). *Anat. Rec.* **22**, 183.

—— (1923). *Journ. Exp Zool.* **37**, 207

—— (1926). *Journ Exp. Zool.* **44**, 419.

TOKURA, R. (1924) *Fol Anat. Japon* **2**, 197.

—— (1925). *Fol. Anat. Japon* **3**, 173.

VOGT, W. (1922 a) *Ver. Anat. Ges.* **31**, 53.

—— (1922 b) *Deut. Med. Woch.* **27**, 926.

—— (1922 c). *Ver. Deut. Zool. Ges.* **27**, 49.

—— (1923). *Ver. Anat. Ges.* **32**, 30.

—— (1925). *Arch. Ent. Mech.* **106**, 542.

—— (1926). *Ver. Anat. Ges.* **35**, 62.

VON UBISCH, L. (1922). *Naturwissenschaften*, **10**, 271.

—— (1924). *Zeit. Wiss. Zool.* **123**, 37.

—— (1925). *Arch Ent. Mech.* **106**, 27.

WACHS, H. (1914). *Arch. Ent Mech.* **39**, 384.

—— (1920). *Sitzber. Ges. Nat. Freunde Berlin*, p. 133.

WARYNSKY, S. and FOL, H. (1884). *Rec. Zool. Suisse*, **1**, 1.

WEBER, A. (1923). *C. Rend. Assoc. Sci. Paris*, **177**, 657.

—— (1924). *C. Rend. Assoc. Anat.* **19**, 287.

—— (1925). *C. Rend. Soc. Biol.* **93**, 592.

WEISS, P. (1925). *Akad. Anzeiger*, Wien, **21/22**, 207.

WERBER, E. I. (1915). *Anat. Rec.* **9**, 529.

WESSEL, E. (1926). *Arch. Ent. Mech.* **107**, 481.

WETZEL, G. (1895). *Arch. Mikr. Anat.* **46**, 654.

WETZEL, R. (1925). *Arch. Ent. Mech.* **106**, 463.

WIEMAN, H. L. (1926). *Journ. Exp. Zool.* **45**, 335.

WILHELMI, H. (1921). *Arch. Ent. Mech.* **48**, 517.

THE COMPARATIVE GENETICS OF COLOUR IN RODENTS AND CARNIVORA

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OUR knowledge of mammalian genetics is practically confined to man, his domestic animals, and four wild rodents. Among these species only the following admit of detailed comparison at present:

Mouse ...	...	*Mus musculus*
"Norway" rat	...	*Mus decumanus*
"Black" rat...	...	*Mus rattus*
Californian deer-mouse		*Peromyscus maniculatus*
Guinea-pig or cavy	...	*Cavia porcellus* and *C. rufescens*
Rabbit ...	...	*Lepus cuniculus*
Dog ...	...	*Canis familiaris*
Cat ...	...	*Felis domestica*
Ferret ...	...	*Martes furo* (and polecat, *Mustela putorius*)

The cow, sheep, goat, pig, horse and man differ considerably in their genetics from the above species and one another, though the horse is perhaps the most similar to the Rodents and Carnivora.

We have reason to believe that genes are definite structures in or on the chromosomes, and we are therefore justified in applying the principle of homology to them, as to other structures. It will be one of the main objects of this paper to determine how this can be done. We cannot, however, with the same propriety apply the term homologous to mutations or to their results. Structures in two species are said to be homologous when they correspond to the same structure in a common ancestor. Now consider the following colours in the mouse and guinea-pig: Agouti ($A B$), Cinnamon agouti ($A bb$), Black ($aa B$), Chocolate ($aa bb$). It is possible that a common ancestor of the rodents acquired the genes A and B in that order. If so, it is perhaps legitimate to call chocolate, black and agouti mice homologous, as each corresponds to an ancestral form. But no common ancestor was cinnamon agouti. In general where homologous allelomorphic pairs of genes are concerned, only $n + 1$ out of the 2^n possible homozygous genotypes can have occurred in ancestors. The following criteria of homology are suggested:

1. Genes are homologous if they produce the same effects when brought in from either side in a species cross. Thus many of the normal genes of *Cavia porcellus* are found in *C. rufescens*. The scope of this criterion could be extended by artificial insemination.

2. The work of Onslow[1] suggests that experiments *in vitro* might yield equally definite results. Since the interaction of skin extracts from different genotypes of a species produces the same results as the crossing of the animals from which they were obtained, the same may be found to be true when the extracts are made from different species.

Homology may be suspected when any or all of the following criteria are satisfied:

3. The genes produce a similar somatic effect. It need not be identical. Thus we can regard E as homologous in rabbits and guinea-pigs. Its absence from a zygote which would otherwise be black leads to yellow young in both cases, but these blacken with maturity in the rabbit, but not in the guinea-pig. Other genes or cytoplasmic differences may account for this divergence.

4. If two different genes with a certain effect are never found within one species we have an additional reason to suspect homology between genes in two different species which produce this effect. Thus, only one gene per species ever converts chocolate into black, but several may convert a piebald into a self-coloured. We therefore homologise with more confidence in the former case than the latter.

5. The genes have undergone several parallel mutations into more or less corresponding multiple allelomorphs.

6. The genes exhibit similar linkages in different species. This condition is not necessary, as shown by the facts relative to the different species of *Drosophila*, summarised by Morgan, Bridges and Sturtevant[2].

The only possible fallacies in criteria (1) and (2) as applied to genes present in a number of species would be as follows:

(*a*) The gene has been independently evolved on several occasions. No instance of the production of homologous dominant genes (other than deficiencies) by mutation has been observed. If it occurs it raises a problem which strikes at the very root of the conception of homology, not only in genetics, but elsewhere.

(*b*) If the common ancestor possessed the gene it might be lost and then re-gained, or duplicated by another of similar function and then lost. These events are perhaps rather unlikely.

It is clear that we have so far no absolute criterion of homology. The question remains whether there may not exist between genes a relation more fundamental than homology, namely, chemical "identity." Linkage results suggest that genes may have a molecular weight of the order of 100,000[2], *i.e.* of the size of protein molecules as found by Adair[3]. If this could be established comparative genetics and ultimately comparative morphology could be placed on a new basis.

But at present we cannot assume such identity between genes which produce indistinguishable effects even in the same species. That a gene may be completely lost is shown by the study of deficiencies of chromosomes or parts of chromosomes in *Drosophila*. But it may presumably become quite inactive without disappearing, and probably in more than one way. It is plausible that, as suggested by Gold-schmidt[4], multiply allelomorphic genes often act by producing varying quantities of the same substance, possibly an enzyme. If the gene found in the wild type is

completely dominant, this means that the effect of two "doses" of the substance produced by a wild-type gene is no greater than that of one. On the other hand, the lower members of the series are generally incomplete dominants, so that here two doses have not the same effect as one.

Now Onslow showed that in the rabbits C and E were both needed for the production of the oxidase which he studied. If each produces a component of this substance the position is somewhat similar to that of zymin, studied by Euler and Myrbäck[5], where either zymase or cozymase, both required for alcoholic fermentation, could be varied simultaneously. Here as the amount of cozymase was increased the rate of fermentation at first increased proportionately to it, and then became constant. If we suppose that the allelomorphs of C produce varying amounts of a component of the oxidase system we should expect the lower members to produce amounts insufficient for complete pigment formation, and therefore to show incomplete dominance and "compound" formation, the higher to be completely dominant. But if so the "normal" genes may produce any quantity of the component above that required for complete dominance, and two such genes need not be identical. It follows that two genes producing the normal effect need not necessarily be chemically identical, even within the same species. In fact, we can apply the term homologous, but no more, to any genes which are allelomorphic with one another, or with homologous genes. An apparent exception to this quantitative conception of allelomorphism is found in the work of Wright[6], who describes two genes C^d which when homozygous cause dilution of black in guinea-pigs, while affecting yellow but little, and its allelomorph C^r which hardly affects black, but abolishes yellow. If we suppose the gene C to be "damaged" differently in the two cases, we can find precise parallels in the field of enzyme chemistry. Thus Myrbäck[7] showed that silver salts depress the activity of saccharase mainly on the alkaline side of its optimum pH, phosphotungstic acid mainly on the acid side. Two allelomorphs may similarly perhaps have different optimum conditions.

With these prolegomena we turn to Table I. The terminology is as far as possible that of Wright[8]. The second column gives the effect on the genotype. As the genes are in general recessive the effect described is that of the gene when homozygous, except in the case of lethals. $+$ means that the gene is present in the normal type, W that it is found in wild races, D that it is found in domesticated animals. Several of the genes marked D have first appeared in wild animals, *e.g.* r and p in rats, but nowhere do they occur in important communities. To economise space, multiple allelomorphs which only correspond roughly, have been classed together. Thus the chinchilla rabbit agrees with the red-eyed dilute guinea-pig in having no yellow pigment, but has a darker eye.

<div align="center">C SERIES.</div>

As we pass down the various series of allelomorphs of C we find a gradual disappearance of both black and yellow coat pigments, and of eye pigments. The dilution on the whole affects yellow more than black. Zygotes with no yellow pigment and much black are known in guinea-pigs and rabbits, but the converse is not found. For this reason Castle and Wright have homologised the recessive

Table I

Gene	Effect	Mouse	Norway rat	Black rat	Deer-mouse	Cavy	Rabbit	Dog	Cat	Ferret
C	Normal	+	+	+	+	+	+	+	+	+
c^k	Slight dilution	−	−	−	−	D	+	−	−	−
c^d	Marked dilution	.	−	−	−	D	+	−	−	−
c^r	No yellow	D	D	−	−	D	D	D	D	−
c^b	Acromelanistic	D	−	−	−	D	D	?D	−	−
c^a	White	D	D	−	D	−	D	−	−	D
A^y	Yellow	D	−	−	−	−	−	?D	−	−
A^w	Light-bellied gray	W	+	+	+	−	+	+	+	−
A^g	Gray-bellied gray	+	−	W	−	+	−	−	D	−
A^r	Ticked-bellied gray	−	−	−	−	W	−	−	D	−
a^t	Black-and-tan	−	−	−	−	−	D	−	−	−
a	Black	D	D	D	−	D	D	D, ?W	D	?+
E^d	Black	.	−	D	−	−	D	−	−	?
E^s	Black	−	−	−	−	−	D	−	−	.
E	Normal	+	+	+	+	+	+	+	+	?+
e^p	Bicoloured	−	−	.	.	D	D	D	−	−
e	Yellow	?D	−	D	D	D	D	D	D	D
b	Cinnamon	D	−	−	.	D	D	D	−	−
r	Red-eyed yellow	−	D	D	D		−	−	−	−
p	Pink-eyed yellow	D	D	−	−	D	−	.	−	−
s_m	Salmon-eyed	−	−	−	−	D	−		−	−
i	Dilute	D	−	D	−	−	D	D	D	−
f	Yellow diluted	−	−	−	−	D	−		−	−
k	"Kodak"	−	−	−	−	D	−	.	−	−
h	Black slightly diluted	?D	−	−	−	−	D	−	−	−
D	Black intensified	?D	−	−	−	−	−	D	D	?+
u	Bicoloured	−	−	−	−	−	−	D	−	−
T^l	Lined tabby	−	−	−	−	−	−		+	−
T^s	Striped tabby	.	−	−	.	−	−		W	−
t^b	Blotched tabby	−	−	.	−	−	.	−	D	.
W	White	.	−	−	−	−	D	D	D	
V	Piebald	D	−	−	−	−	D	D	?D	.
s_1	Piebald	D	D	−	.	D	D	D		.
s_2	Piebald	−	.	−	−	−	D	D	−	−
s_3	Piebald	−	−	−	.	.	D	.	−	.
s_4	White nose, feet or tail	D	−	−	D	−	D	D	−	−
R_o	Roan	−	−	−	−	D	?D	D	−	−
s_t	Silvered	?D	.	−	−	−	?D	.	.	−

genes responsible for the "albino" dog which contains some pigment, and the Siamese cat. The latter has blue eyes and is born white, but darkens all over and exhibits marked acromelanism. The albino cocker spaniels are acromelanic like the Himalayan rabbit and the adult albino guinea-pig. The albino Pekinese, on the other hand, have some pigment all over if they carry the gene D (Pearson, Nettle-

ship and Usher's "lilacs"). Hence, there are probably two different genes in this series in the dog. The lowest allelomorphs in guinea-pigs and dogs, and the lowest but one in mice and rabbits, exhibit acromelanism, which Schultz[9] has shown to be conditioned by cold, any area of the skin which is sufficiently cooled down producing black hair.

<center>*A* SERIES.</center>

The genes of this series produce a substance which inhibits the formation of black or chocolate pigments. Except perhaps in the guinea-pig, it may also cause some inhibition of yellow. The inhibition of black on the dorsal surface is periodic in the normal type, so that one or more yellow portions are found in each hair. On the ventral surface almost all the black, and generally much of the yellow, is inhibited. In mice A^y in two doses is lethal at an early prenatal stage. In one dose it produces a yellow. The colour may be uniform, or the belly may be light-coloured. The A^y gene is also found in the various types of sable, black-and-tan, chocolate-and-tan, etc., mice. Their genetical behaviour is still far from clear, nor is it obvious why a yellow mouse should often, though not always, have a lighter back, but darker belly than the wild type. The case of C^r and C^d in the guinea-pig perhaps affords a parallel. In short-haired Dachshunds a yellow form is dominant over both agouti and black-and-tan. As no black inhibitors other than A series are known in the rodents, I have tentatively homologised the gene causing it with A. The evidence is insufficient to determine whether it shows the "complete repulsion" from the agouti gene to be expected if this homology is correct.

In mice both white-bellied and gray-bellied agoutis exist, due to two different genes of this series. Similarly the yellow-bellied agouti of *Cavia porcellus*, and the agouti-bellied agouti of *C. rufescens* are due to members of it. I therefore venture to suggest that the difference between *Mus rattus* var. *alexandrinus* and var. *tectorum*, which resemble the two mouse types, is due to the same cause. This cannot be proved until a recessive black appears in this species[1]. The black-and-tan rabbit, with black back and yellow belly, is due to a still lower allelomorph. The lowest member of the series, black, is found in most of the species. In the cat there are three different types of banding. They appear to be caused by three multiple allelomorphic genes, that causing the greatest inhibition of black being dominant over the other two, the intermediate over the least effective, and all three over black. The evidence is, however, not quite conclusive. In the dog, however, black has not been shown to be recessive to agouti. But in Dachshunds agouti is dominant over black-and-tan, which is here recessive to black (and therefore does not correspond to the black-and-tan of mice or rabbits). As A^y in the Dachshund inhibits black in the black areas of the coat, it would probably do so in the black also. Both black and agouti are found in wild foxes, but no data are available on their inheritance. The gene A may be absent in the ferret and polecat, or merely inhibited.

Two interesting points emerge as to the A series. In the first place, the normal gene may vary upwards as well as downwards, as is probably the case with E in the rabbit, and certainly in *Primula sinensis*. Here, as Gregory, de Winton, and Bateson[10]

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[1] Feldman has since proved it (*Genetics* 11, p. 457, 1926).

showed, the white eye of "Queen Alexandra," a dominant over the normal, which
has appeared in Europe since 1821, is multiply allelomorphic with the normal and
the recessive large yellow eye of "Primrose Queen." In "Queen Alexandra" the gene
in the normal which suppresses the yellow pigment has been intensified just as the
suppression of black has been intensified in the yellow mouse. In the second place,
the gene varies in nature, though the variations are less marked than those which
were first analysed by Mendelians. But the facts with regard to *Cavia* render it
certain that mutation of this gene has played some part in the evolution of that
species. The facts with regard to the mouse and Black rat render it probable that
in them the early stages of species formation can be observed.

<div align="center">E SERIES.</div>

In certain black rabbits there exists a gene E^d which counteracts A^w. E^d has
no visible effect in the absence of A. If one or two A's are present E^dE^d and E^de
animals are black, whilst E^dE are black with a few agouti hairs. It is possible that
E^d gives rise to an amount of the normal product of E, too large for A to inhibit.
It may be of course that E^d is really composed of a gene D very closely linked to E.
As, however, Punnett obtained no cross-overs in 476, this is rather improbable.
On this hypothesis, however, we should expect E^dE rabbits to be intermediate
between E^dE^d and E^de, *i.e.* black, which is not the case. They possess a small area
over which the E gene exerts some effect. This curious fact may perhaps be cor-
related with the tendency of the E series to express itself by patches of different
colours rather than by blending. Onslow describes a very similar gene of which
two doses added to an agouti produce blacks, one "steels," or dark type of agouti.
Castle, apparently working with this gene rather than E^d, found complete coupling
with E. I have therefore ventured to describe Onslow's gene as E^s.

A multiple allelomorph e^p of this series giving black-and-yellows is found in the
guinea-pig, rabbit and dog. Here a weakening of the gene shows itself not as with
C and A, in a general dilution of the effect, but in a complete failure to function
over certain areas. This "all-or-none" reaction is far harder to explain on chemical
lines than those of the A and C series, though one case more or less analogous has
been described by Eadie [11] in the field of enzyme chemistry. I shall return to it
when considering the tortoiseshell cat. e^p in the rabbit inhibits A over the black
areas, whereas A acts fully on the yellow areas, the so-called Japanese (e^pe^p and
e^pe) rabbits having black patches on a yellow or tortoiseshell ground according as
A is or is not present. In other words, on a given area of skin, e^p either produces
no effect whatever, or that of E^d. The analogy with the case of c^d and c^r is obvious.
Recessive yellow has only been described by Hagedoorn in the mouse, and requires
confirmation. In *Mus rattus* and *Peromyscus* these are black-eyed creams probably
homologous with the brighter yellows of the other species. In the dog black is
dominant over yellow in most breeds. In all cases the eye of ee animals is normal.
ee rabbits are born yellow, but darken with age into "tortoiseshells" unless the
black pigment is inhibited by A. In the cat E is sex-linked, but as in *Drosophila*
apparently homologous genes may be sex-linked in one species but not another,

this does not here exclude homology with the rodents. $E\ E$ ♀ and E ♂ cats (with rare exceptions) are black, Ee ♀ black and yellow (tortoiseshell) and ee ♀ and e ♂ yellow. These facts are hardly compatible with the presence-and-absence theory in its original form, for it is clear that the presence of e converts the black into a black and yellow. In the cat the second black factor D is also incompletely dominant over yellow, giving tortoiseshell of both sexes when heterozygous, so that the conditions in cats in general seem to favour the development of this pattern. The only reason for homologising the sex-linked rather than the autosomal blackening gene with that of rodents is that the former appears to be present in the wild cat. It is however strictly neither dominant nor recessive and the homology is dubious.   In the ferret yellow is recessive to black.

B.

In the absence of this gene the black pigment of the wild type is replaced by chocolate in the coat, and the eyes may be a dull red. Dominance is complete except in two cases. In mice lacking A and P it is possible to distinguish BB (blue lilac) from Bb (chocolate lilac). Similarly rabbits of constitution $E^dE\ Aa\ BB$ and $E^dE\ Aa\ Bb$ are noticeably different, the latter having more and lighter agouti hairs. In the guinea-pig A seems to act rather more effectively in the absence of B, for AA bb and $Aa\ bb$ animals commonly have a sprinkling of almost white hairs.

R.

In both species of rat and in *Peromyscus* a red-eyed yellow type is known. Their eye colour is distinctly darker than that of pp or cc animals, and becomes almost black in old *rattus* individuals. Although ee animals are black-eyed one might be tempted to homologise R with E but for two facts. It is very closely linked with C, where this can be tested, while in Hagedoorn's recessive yellow mice no such linkage was found, and black-eyed yellows are known in both *Mus rattus* and *Peromyscus*, which I have ventured to homologise with the ee yellows of other species.

P.

In the absence of this gene black and chocolate pigments are largely but not completely absent in the coat, and perhaps wholly so in the iris. The eye is therefore pink, the coat yellowish. The linkage with C makes the homology of P in the mouse and rat obvious. The linkage is not found in the guinea-pig, but homology is probable.

S_m.

In the guinea-pig the absence of this gene gives a pink-eyed individual with practically normal coat colour. The iris may or may not carry a ring of pigment near the pupil.

I.

In the absence of I, black, chocolate and yellow are diluted. However in yellow and sable mice dilution may be due to other causes. Homology is not certain.

F.

In the absence of this gene, yellow, but apparently not black or chocolate, is diluted. Its effect is thus not unlike that of C^r, but weaker. Possibly identical with F, but probably not, is the gene causing a fading of the yellow, but not the black in $c^d c^a$ guinea-pigs. The colour is present at birth but later disappears. The gene concerned may perhaps be the same as F. If not it is at present uncertain whether it has any visible effect in the presence of the genes of the wild type, or is merely a modifier in Morgan's sense, that is to say, effective only on an abnormal genotype.

K.

In the absence of K, guinea-pigs of composition $e^p e^p pp$ or $e^p e pp$ are born without black or chocolate pigments, which develop later. It is not known what effect, if any, is produced on other genotypes.

H.

This gene has been described in mice and rabbits. In its absence black or chocolate pigment is somewhat diluted. In mice it is stated by Hagedoorn to show its effects mainly on agoutis and chocolates, and to be absent in many wild individuals. The latter phenomenon may perhaps be really the substitution of A^w for A^y, but this cannot account for the effect in chocolate individuals. The whole question cannot as yet be regarded as settled. In rabbits H has apparently been lost in certain chocolate and blue races. As its effects are more notable in varieties than in the wild form, it may be regarded as a "modifier" in Morgan's sense.

D.

Several cases are known where the effect of A is wholly or partially inhibited by a dominant gene. In mice incompletely analysed causes convert the yellow into a sable or black-and-tan. These seem to be Mendelian genes as there is increased variability and some degree of segregation in F_2 from crosses of black-and-tan with ordinary mice. As the segregates are of the normal yellow and agouti types and breed true, the darkening genes must be on the whole dominant. On the other hand, single genes more or less completely inhibiting A are known elsewhere. The "black" *Mus rattus* differ by one such from the gray races. This may, of course, turn out to be homologous with the E^d of rabbits[1]. In the dog a gene inhibiting A is also known, which may also prove to be E^d. In cats a gene D suppressing A is found in the Siamese race. It is autosomal and hence cannot be an allelomorph of the gene (here perhaps wrongly homologised with E) which distinguishes the ordinary black from the yellow cat. When Siamese are mated with yellow, the F_1 are tortoiseshell in both sexes. Clearly a complete interpretation of these phenomena is still lacking.

U.

In dogs black-and-tan, chocolate-and-tan, and red-and-tan are recessive to black, chocolate, and red respectively. The effect of the absence of U is therefore

[1] Feldman has since shown this to be the case.

similar to that of the absence of I, save that it takes effect only over certain areas (*e.g.* spots on the eyebrows). It seems unlikely that there is any homology with a^t of rabbits.

$$T^l, T^s, t^b.$$

These genes, whose multiple allelomorphism is not as yet conclusively proven, determine the tabby pattern in the cat. The dark stripes are black on a gray ground in presence of A and E, slightly blacker than their surroundings in the absence of A, and orange on a yellow ground in the absence of E. The pattern is always present, but in cats bearing T^l the stripes are narrower, in $T^s T^s$ and $T^s T^b$ they are broader, in $t^b t^b$ they have degenerated into blotches. T^l appears to be found in the Abyssinian, T^s in the European wild cat.

$$W.$$

Completely white forms are found in the rabbit, dog and cat which are dominant over the normal type. The eyes are generally blue, *i.e.* more pigmented than those of the albino, and dominance is generally rather imperfect. In the rabbit W is probably weakly linked with V, and hence with L, the gene whose absence causes long hair. In the cat W is also possibly linked with L. If these linkages are proved an argument will clearly be presented for the homology of W in the rabbit and cat.

$$V.$$

Dominant piebalds are found in the mouse, rabbit and dog. In the former the gene is lethal. In the rabbit it determines a number of black spots on a white ground. In the dog this pattern is produced in Great Danes and Dalmatians, but elsewhere dominant piebalds usually have fairly large continuous areas. The different types of piebaldness may be due to modifiers, multiple allelomorphism, or several genes. When the spotted and unspotted types of white dog are mated the heterozygotes are intermediate. In an F_2 of 6 all were piebald. This does not, of course, exclude the possibility of two independent dominant genes. In the cat piebaldness is probably dominant.

$$S_1, S_2, S_3, S_4.$$

In the mouse one recessive gene is known giving piebalds, another giving white hair on the nose and occasionally the chest. Calling the first of these S_1, $Vv s_1 s_1$ mice are black-eyed white. In the Norway rat the two main types of piebaldness, "Irish" and "hooded," are due to multiple allelomorphs according to Castle, but not according to Doncaster. Modifying genes undoubtedly exist. In *Peromyscus* recessive piebaldness may be confined to the tail or spread to the belly. In the cavy piebaldness is recessive. In the rabbit it appears necessary to postulate four recessive (but not completely recessive) genes concerned in piebaldness. According to Castle two of these are allelomorphs of one another, which is disputed by Punnett and Pease. The arguments are too lengthy to summarise. There seems no doubt that the principal gene S_1 (Castle's D_u, Punnett and Pease's P) is linked with the gene L causing short hair, with V and probably with W. Of the other genes one

at least appears to give animals with white feet and nose when in the recessive condition, but their effect is more striking in the absence of the principal gene S_1. As a result of various combinations rabbits appear of all grades from self-coloured to almost white. In the absence of S_1 (P) and then only, the iris may be wholly or partly blue. In the dog recessive piebalds exist, and also recessives with white feet and muzzles. It is not known whether the two genes concerned have an additive effect. A gene (here called S_2) is also known such that $S_2 S_2$ is self-coloured, $S_2 s_2$ dappled, containing white and coloured hairs mixed, while $s_2 s_2$ animals are dappled with white patches. This last type exhibit eye and other defects and is unhealthy, in fact, the gene is semi-lethal.

The type of pattern produced is on the whole characteristic of the species and not the gene. Piebald mice and guinea-pigs generally have an asymmetrical pattern, while rats and rabbits are more symmetrical. But the individual genes have slightly different effects. Thus the dominant lethal V of the mouse gives less sharply marked patches than the recessive $s_1 s_1$. And in the rabbit the absence of S_1 (P, D_u) causes a more regular pattern than the combined effect of the absence of S_2 and S_3 (P and T). There can therefore be no question at present of homologising any of these genes in different species.

$$R_n.$$

In the cavy a gene causes silvering in chocolates, and to a much less extent in blacks. It is incompletely dominant. Full details are not yet available. In the rabbit silver × normal gives intermediates. In the dog roan appears also to be dominant, though it is not yet absolutely certain that the genes R_n and V are distinct.

$$S_r.$$

Recessive silvering, not present at birth, has been described in the mouse, and a recessive gene may be present in some silver rabbits.

LINKAGE.

The main facts are given in Table II. It will be seen that the linkage is stronger in the male or heterozygous sex as pointed out elsewhere[12]. L is the gene causing short as opposed to long hair. The result for the cat has little significance. The linkage between V and S_1 in the rabbit is very close, but one cross-over has been obtained.

Three cases of linkage in a single individual have been noted. Hagedoorn mentions a case which he describes as repulsion of A^g and C, but his facts do not agree with this or any other simple hypothesis. Castle had an $Ii \, Vv$ rabbit which gave 32 cross-overs out of 83 young, with $ii \, vv$'s, a cross-over value of $38 \cdot 5 \pm 3 \cdot 7$, while others have given $51 \cdot 1 \pm 1 \cdot 4$. Dunn obtained a linkage value of $34 \cdot 5 \pm 3 \cdot 4$ in one family between A^v and B. These genes were repelled in the first generation tested, and apparently coupled in one of their offspring. They are not normally linked. These cases may be explained as due to a temporary union between two chromosomes, or

Table II

Animal	Genes	C.O.V.	P.E.
Mouse ♂	C P	12·00	0·22
„ ♀	C P	16·06	0·27
Norway rat ♂	C R	0·18	0 03
„ ♀	C R	0·53	0·08
„ ♂	C P	18·39	0·26
„ ♀	C P	21 93	0·39
„ ♂	R P	15·55	0·44
„ ♀	R P	20·46	0·53
Peromyscus	C R	2·8	2·0?
Rabbit	C B	36·1	1·3
„	V S	0·14	0·10
„	W L	43·4	2·0
„	V L	13·0	0·8
„	S L	14·3	0·9
Cat	W L	0	23

less probably to a translocation of a part of one on to another. Unfortunately they have not been followed up adequately.

Except that C and P are in the same chromosome in the rat and mouse, C and R in the rat and *Peromyscus*, little can be made of these data from a comparative point of view. A fair amount of negative data as to linkage exist, but unless repeated on a larger scale are not quite conclusive. Thus in the mouse P and S_1 give a c.o.v. of $46·84 \pm 0·97$, which may be significant, but would require much further work to establish. It is striking that V and S are quite independent in the mouse, but, as pointed out above, there is no great reason to suppose that either of these genes is homologous in the rabbit and mouse.

CONCLUSIONS.

At least twenty different genes for colour exist in the nine animal species considered, or counting multiple allelomorphs, at least thirty-six. Of the mutations which have occurred from the wild type seven are dominant or semi-dominant, and two of these are lethal. At least seventy recessive mutations have occurred. The dominants exhibit little similarity in different species. The dominant yellow of mice is lethal, that of dogs is not. The dominant piebald of mice is lethal, that of rabbits is not. The dominant and semi-dominant blacks of the rabbit may have homologies in the black rat[1] and dog, but this is not certain. The same applies to the dominant whites. We cannot say for certain that the concept of homology is applicable to dominant mutations. There is, on the other hand, no doubt at all that it applies to recessives. We may assume that the wild type of all the species considered (the wild type of ferret being the polecat, with which it breeds, and which certainly possesses C, and almost certainly E, except in local races) contains C, A,

[1] Feldman has since proved it for the rat.

E, B, R, P, I, S_1, S_2, and probably S_m, F, K, H, S_3, S_4, and S_i. We can be fairly certain of the homologies of the first six of these. C has mutated in eight, A in seven, E in eight, B in four, R and P in three species each. On the other hand, S_m, F and K have only mutated in one. It appears therefore that there is a real difference in the tendency of different genes to mutate. On the other hand, the data available do not show that any given gene has a special tendency to mutate in one particular species or group of species. They rather suggest a fundamentally similar germinal composition liable to similar modifications in all the species.

From the point of view of evolution the most interesting fact is that a *small* mutation of the gene A has certainly occurred in nature in the cavy, mouse and black rat. E^d is found in the northern race of *Mus rattus*, and there is a probability that the Abyssinian and European wild cats possess T^i and T^s respectively. Those who deny the importance of Mendelian genes in evolution point to the absence of clear-cut segregation in many crosses between species and local races. The data for the species under consideration suggest that evolution has occurred, to some extent at least, by slight changes in the intensity of action of genes. Even if such minor factors as the difference between A^w and A^y could be isolated in a species cross, it would be very difficult to determine their nature unless the gene under consideration had mutated to a greater extent in one of the species concerned, and indeed in *Mus rattus* the nature of the difference between *tectorum* and *alexandrinus* was only determined while this paper was in the press.

To sum up, mutation of colour-determining genes in the species considered is a more or less orderly process. It shows the essentially similar character of their genetical composition, and throws some light on their evolution.

REFERENCES.

(1) ONSLOW (1915). *Proc. Roy. Soc.* B, **89**, 36.
(2) MORGAN, BRIDGES and STURTEVANT. *Bibliog. Genet.* **2**, 1.
(3) ADAIR (1924). *Biol. Proc. Camb. Phil. Soc.* **1**, 75.
(4) GOLDSCHMIDT (1923). *Arch. Mik. Anat.* **98**, 192.
(5) EULER and MYRBÄCK (1924). *Zeit. Physiol. Chem.* **136**, 109.
(6) WRIGHT (1925). *Genetics,* **10**, 223.
(7) MYRBÄCK (1926). *Zeit. Physiol. Chem.* **158**, 162.
(8) WRIGHT (1917, 1918). *Journ. Hered.* **8, 9**.
(9) SCHULTZ (1921, 1922). *Arch. Entw.* **41, 42, 51**.
(10) GREGORY, DE WINTON and BATESON (1923). *Journ. Genet.* **13**, 219.
(11) EADIE (1926). *Biochem. Journ.* **20**, 1016.
(12) HALDANE (1922). *Journ. Genet.* **12**, 101.

C SERIES.

Mouse. CUÉNOT (1902). *Arch. Zool. Exp. et Gen.* **3**, 10, xxvii.
     DETLEFSEN (1922). *Am. Nat.* **56**, 573.
     FELDMAN (1921). *Am. Nat.* **55**, 470.
Norway rat. BATESON (1903). *Proc. Zool. Soc.* **2**, 71.
     WHITING and KING (1918). *Journ. Exp. Zool.* **26**, 55
Peromyscus. SUMNER (1922). *Am. Nat.* **56**, 412.
     CASTLE (1912). *Science,* **35**, 346.
Guinea-pig. SOLLAS (1909). *Rep. Ev. Ctee Roy. Soc.* **5**, 51.
     WRIGHT (1925). *Genetics,* **10**, 223.

Lepus. PUNNETT (1912). *Journ. Genet.* **5**, 37.
    CASTLE (1921). *Science*, **53**, 387.
Conis. PEARSON, NETTLESHIP and USHER (1913). *A Monograph on albinism in man*, Part 2, p. 460.
Ferret. PITT (1921). *Journ. Genet.* **11**, 99.

A SERIES.

Mouse. CUÉNOT (1902–1910). *Arch. Zool. Exp. et Gen.* **4** (2), p. xxxiii; **4** (2), p. xxiii; **4** (2), p. xlv;
    4 (3), p. cxviii; **4** (6), p. i; **4** (7), p. vii.
    LITTLE (1919). *Am. Nat.* **53**, 185.
Norway rat. BATESON (1903). *Proc. Zool. Soc.* **2**, 71.
Black rat. BONHOTE (1915). *Vigour and Heredity*, p. 64. *Proc. Zool. Soc.* (1910), p. 651.
Guinea-pig. SOLLAS (1909). *Rep. Ev. Ctee Roy. Soc.* **5**, 51.
    WRIGHT (1916). *Carn. Inst. Wash. Pub.* **241**, 59.
Rabbit. HURST (1905). *Linn. Soc. Journ.* **29**, 283.
    CASTLE and FISH (1915). *Am. Nat.* **49**, 88.
Dog. ANKER (1925). *Biol. Med. Rgl. Vid. Selsk.* **4**, 6.
Cat. DONCASTER (1904). *Proc. Camb. Phil. Soc.* **13**, 35.
    WHITING (1918). *Journ. Exp. Zool.* **25**.
    —— (1919). *Am. Nat.* **53**, 473.

E SERIES.

Mouse. HAGEDOORN (1911). *Zeit. Ind. Ab. u. Ver.* **6**, p. 104.
Black rat. CREW (1923). *Journ. Hered.* **14**.
Peromyscus. SUMNER (1922). *Am. Nat.* **56**, 412.
Guinea-pig. SOLLAS (1909). *Rep. Ev. Ctee Roy. Soc.* **5**, 51.
    IBSEN (1919). *Genetics*, **4**, 597.
Rabbit. PUNNETT (1924). *Journ. Genet.* **14**, 231.
    —— (1915). *Journ. Genet.* **5**, 37.
    ONSLOW (1922). *Journ. Genet.* **12**, 91.
    CASTLE (1922). *Carn. Inst. Wash. Pub.* **320**, 26.
Dog. LITTLE (1914). *Journ Hered.* **5**, 244.
    LITTLE and JONES (1919). *Journ. Hered.* **10**, 309.
Cat. DONCASTER (1904). *Proc. Camb. Phl. Soc.* **13**, 35.
Ferret. PITT (1921). *Journ. Genet.* **11**, 99.

B.

Mouse. DURHAM (1908). *Rep. Ev. Ctee Roy. Soc.* **4**, 41.
Guinea-pig. SOLLAS (1909). *Rep. Ev. Ctee Roy. Soc.* **5**, 51.
Rabbit. PUNNETT (1912). *Journ. Genet.* **2**, 221.
Dog. LITTLE (1914). *Journ. Hered.* **5**, 244.

R.

Norway rat. CASTLE (1916). *Carn. Inst. Wash. Pub.* **241**, 175.
Black rat. BONHOTE (1915). *Vigour and Heredity*, p. 69.
Peromyscus. SUMNER (1922). *Am. Nat.* **56**, 412.

P.

Mouse. DURHAM (1911). *Journ. Genet.* **1**, 159.
Norway rat. CASTLE (1910). *Carn. Inst. Wash. Pub.* **241**, 175.
Guinea-pig. WRIGHT (1910). *Carn. Inst. Wash. Pub.* **241**, 59.

$S_m.$

Guinea-pig. IBSEN (1924). *Anat. Rec.* **29**, p. 140.

I.

Mouse. DURHAM (1909). *Rep. Ev. Ctee Roy. Soc.* **4**, 41.
Rabbit. CASTLE (1909). *Carn. Inst. Wash. Pub.* **114**.
Dog. LITTLE and JONES (1919). *Journ. Hered.* **10**, 309.
Cat. DONCASTER (1904). *Proc. Camb. Phil. Soc.* **13**, 35.

F.

Guinea-pig. WRIGHT (1923). *Am. Nat.* **57**, 42.
    —— (1925). *Genetics*, **10**, 223.
    IBSEN (1925). *Anat. Rec.* **31**, 355.

K.

Guinea-pig. IBSEN (1925). *Anat. Rec.* **31**, 355.

H.

Mouse. HAGEDOORN (1912). *Zeit. Ind. Ab. u. Ver.* **6**, 97.
Rabbit. KOSSWIG (1925). *Zeit. Tierzucht*, **5**, 119.

D.

Mouse. DUNN (1920). *Am. Nat.* **54**.
Black rat. MORGAN (1909). *Am. Nat.* **43**, 182.
Dog and Cat. TJEBBES and WRIEDT (1926). *Journ. Genet.* **17**, 207.

U.

Dog. BARROW and PHILLIPS (1915). *Journ. Hered.* **6**, 387.

T^l, T^s, t^b.

Cat. WHITING (1918). *Journ. Exp. Zool.* **25**.
—— (1919). *Am. Nat.* **53**.

W.

Rabbit. CASTLE (1924). *Journ. Hered.* **15**, 211.
Dog. ONSLOW (1923). *Biochem. Journ.* **17**, 334.
Cat. TJEBBES (1924). *Journ. Genet.* **14**, 355.

V.

Mouse. LITTLE (1919). *Am. Nat.* **53**, 727.
Rabbit. CASTLE (1915). *Am. Nat.* **49**, 23.
Dog. LITTLE and JONES (1919). *Journ. Hered.* **10**, 307.
Cat. WHITING (1919). *Am. Nat.* **53**, 473.

S_1, S_2, S_3, S_4.

Mouse. DURHAM (1908). *Rep. Ev. Ctee Roy. Soc.* **4**, 49.
    LITTLE (1914). *Am. Nat.* **48**, 74.
Norway rat. DONCASTER (1905). *Proc. Camb. Phil. Soc.* **13**, 212.
    MACCURDY and CASTLE (1907). *Carn. Inst. Wash. Pub.* **70**.
Peromyscus. CASTLE (1924). *Genetics* and *Eugenics*, 162.
Guinea-pig. IBSEN (1916). *Genetics*, p. 287.
Rabbit. PUNNETT and PEASE (1925). *Journ. Genet.* **15**, 375.
    —— —— (1926). *Journ. Genet.* **16**, 197.
    CASTLE (1926). *Journ. Genet.* **16**, 189.
Dog. WRIEDT (1925). *Zeit. Tierzucht*, **3**, 223.

R_n.

Guinea-pig. IBSEN (1925). *Anat. Rec.* **31**, 355.
Rabbit. MARCHLEWSKI (1924). *Bull. Ac. Polon. Sci. et Lett.* B, 697.
    PAP (1921). *Zeit. Arch. Ind. Ab. u. Ver.* **26**, 185.
Dog. BARROW and PHILLIPS (1915). *Journ. Hered.* **6**, 387.

S_i.

Mouse. HAGEDOORN. *Zeit. Ind. Ab. u. Ver.* **6**, 104.
Rabbit. MARCHLEWSKI (1924). *Bull. Ac. Polon. Sci. et Lett.* B, 697.

GENERAL REVIEWS.

WRIGHT (1917, 1918). *Journ. Hered.* **8**, 9.
KOSSWIG (1925). *Zeit. Tierzucht*, **5**, 119.
CASTLE (1924). *Genetics* and *Eugenics*.

LINKAGE.

Mouse and Rat. KRÖNING (1924). *Jahresber. Ges. Physiol.* **5**, 66.
    CASTLE and WACHTER (1924). *Genetics*, **9**, 1.
    DUNN (1920). *Genetics*, **5**, 344.
Peromyscus. SUMNER (1922). *Am. Nat.* **56**, 412.
Rabbit. CASTLE (1925, 1926). *Ann. Rep. Carn. Inst. Wash.*
Cat. TJEBBES (1924). *Journ. Genet.* **14**, 355.

THE VERTICAL DISTRIBUTION OF PLANKTON IN THE SEA

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(With Eight Text-figures.)

CONTENTS.

INTRODUCTION.

THE object of this article is to present, as far as possible, a full account of our knowledge of the vertical distribution in the sea of those animals and plants which exist, either temporarily or permanently, as "drifters" in the water layers between the surface and the bottom: to collect information on the various factors that exist in nature which may influence their distribution: and to summarise the many theoretical suggestions, arising either from observations of natural phenomena or from the results of experimental work in the laboratory, which have been put forward from time to time, essaying to define the connection between the natural

behaviour of the animals and plants of the plankton and the various external and internal factors that form their environment.

The paper is set out on the following plan:

1. Information of the various external factors that may or may not be of importance in controlling the vertical distribution of plankton organisms.

2. A general survey of our knowledge of the vertical distribution of plankton plants and animals in the sea, and the theories suggested by field observations.

3. The principal results obtained by experimental work, and the theoretical suggestions arising from them.

Although the work is concerned primarily with the behaviour of the zoo-plankton, a short account of the vertical distribution of the phytoplankton is inserted to make the picture complete.

A full account of our knowledge of the actual behaviour of the animals in nature is here given before any allusion to experimental work. It is hoped that this may emphasise the point that a study of behaviour in the field should always precede laboratory experiments. Without a sound knowledge of the animal's behaviour under its normal surroundings the interpretation of laboratory reactions becomes extremely difficult and, very often, misleading.

A fairly full bibliography is given at the end, but it has been impossible to include all observations on vertical distribution. Such would involve giving full lists of all the reports on plankton organisms collected by the numerous oceano-graphical expeditions. As far as possible, however, all those papers are included in which the subject has assumed an important aspect.

POSSIBLE CONTROLLING FACTORS IN THE VERTICAL DISTRIBUTION OF PLANKTON.

The following factors and their changes with respect to depth must be examined for a proper understanding of the vertical distribution of plankton in the sea:

Physical.
> Light intensity and colour.
> Temperature, viscosity and density.
> Currents and wind effects.
> Pressure.

Chemical.
> Salinity.
> Oxygen and CO_2 content, dissolved nutrient salts, etc.

In addition it is necessary to know the possible swimming speeds of the various animals and the rate of sinking of both plants and animals.

Light.

The light conditions in the sea will here be dealt with at some length, because, as we shall see later, this factor is probably one of very great importance.

Light is absolutely necessary for the life of all the floating plants; without light, as far as we know at present, the assimilation of the CO_2 dissolved in the surrounding medium with the aid of chlorophyll would not be possible. "Continued existence only becomes possible for plants when the light is of such an intensity that it enables photosynthesis to be carried out at a rate at least equal to that at which the carbon compounds of the plants are dissipated through respiration" (Atkins, 1926, *b*, p. 100).

At the same time the actual composition of the light is of importance since rays of certain wave-lengths are more effective in aiding photosynthesis in some plants than in others.

That light is an important factor in the behaviour of animals is well known; it has also been shown that some animals will react to one wave-length more than another.

It is therefore of importance that we should know to what depths light penetrates in the sea and how it varies in intensity and composition at different depths.

In pure water, or in water in which the coefficient of absorption of light is the same at all depths, the intensity decreases from the surface downwards in geometrical progression. That is, if the intensity at a depth of one metre is half of that just at the surface, at two metres it will be half of that at one metre. The coefficient of absorption for each wave-length differs, the red rays being absorbed quickest, and the green, blue and violet penetrating deepest. Little is as yet known of the actual intensities and compositions of light at different depths in the open sea. The presence of light has, however, been demonstrated at great depths in the Sargasso Sea (Helland-Hansen in Murray and Hjort, 1912, p. 251) and in the Mediterranean (Grein, 1914) by photographic methods. Recently advances have been made by means of photo-electric cells (Poole, 1925; Poole and Atkins, 1926, and Shelford and Gail, 1922), but few actual readings are as yet forthcoming. For summaries of our present knowledge and full bibliographies see Atkins (1926, *b*), and Klugh (1925, *b*).

The factors that affect the intensity of the light beneath the sea surface are:

1. The actual intensity in air; this varies from season to season and from hour to hour.

2. The altitude of the sun.

3. The state of the sea surface; a glass-calm sea allows the greatest penetration. If the surface is ruffled there is a large loss by reflection.

4. Turbidity of the water due to fine particles in suspension and the presence of plankton organisms. The effect on the transparency of sea water produced by plankton organisms has been demonstrated by Lohmann (1908, p. 229) with the aid of a Secchi disc—a white disc used to measure the transparency by lowering it to the depth at which it disappears. This depth limit varies from place to place and from time to time at the same place.

A few observations are:

Laboe in Kiel Bay	Maximum, 9 m.	—
	Minimum, 2 m.	(Lohmann, 1908, p. 230)
Sargasso Sea	58 m. and 66 m.	(Krümmel, 1893, p. 100)
Western Baltic	16 m.	—
Clyde sea area	22 m.	—
Mediterranean	54 m.	—
Red Sea	21 m.	(Thoulet, 1905, p. 115)
English Channel (Plymouth)	9 to 20 m.	(Russell, unpublished)
Gulf of Maine	5·4 to 15 m.	(Bigelow, 1914, p. 82)
Tortugas {Lagoon Water	7·5 to 12·7 m.}	(Taylor, 1925-26)
{Gulf Stream Water	25·5 to 36·4 m.}	

It may be stated as a general fact that the water becomes more transparent the farther one goes from the coast, the blue of the open oceans being a sign of great transparency. The warm, more equatorial seas are generally more transparent than the cold arctic waters which may be at times so thickly populated with Diatoms and other plankton organisms as to have a green coloration.

It will be convenient here to quote some actual figures of light intensities at different depths, as a knowledge of such is of the greatest importance in realising the significance of the behaviour of many plankton organisms.

Poole and Atkins (1926, p. 192). Photo-electric cell with maximum sensitivity for blue light used	Time	Depth in metres	Intensity in metre-candles		Percentage of air-illumination
			Air	Water	
1. x. 25. E 1. Ten miles S.W. of Eddystone Lighthouse, in English Channel. Depth, 72 m. Light air, N.N.W. Very slight oily swell; glassy surface. Dull to weak sun	2.11 p.m.	Air	22,400	—	100
	1.58 ,,	1 5	21,100	15,000	71·2
	1.46 ,,	6 1	22,500	9,280	41·2
	1.18 ,,	8·9	20,400	5,770	28·3
	12.53 ,,	12·2	21,300	3,970	18·6
	12.40 ,,	18·3	18,300	1,450	7 92
	12.31 ,,	24·4	16,000	470	2·93
	12.19 ,,	34·8	17,400	94	0·54
3. ix. 25. Cawsand Bay. Depth, 10·4 m. Fresh N.W. wind causing waves to break. No swell. Bright sun	10.31 a.m.	Air	68,700	—	100
	10.23 ,,	2·35	74,000	37,800	51·1
	10.11 ,,	4·35	70,300	25,800	36·7
	9.43 ,,	5 9	64,800	19,800	30·5
	10.5 ,,	6·5	69,300	19,000	27·4
	10.0 ,,	8 3	66,100	14,100	21·3
	9.54 ,,	9·85	66,100	11,500	17·4
	9.50 ,,	10·35	65,600	7,420	11·3

These figures show how very rapidly the light falls off in intensity in inshore turbid waters compared with offshore waters, the percentage illumination having fallen to 21·3 per cent. at 8·3 m. in Cawsand Bay, while at 8·9 m. at E 1 it was still 28·3 per cent. On this occasion, with an offshore wind, the water in the bay was comparatively clear, but Poole and Atkins (1926, p. 191) give a further observation showing how on another day in Cawsand Bay the light intensity had already fallen to 9·5 per cent. at a depth of 8·1 m.

At the same time the figures show very well how the *actual* intensity varies from day to day, being about three times as great on a day with bright sun as on a dull day at that time of year.

Klugh (1925, *b*, p. 230) gives for the Bay of Fundy the following figures:

Aug. 3rd, 1924. Bright sun. Clear sky. Smooth surface.

2 cm. below surface* (taken as standard)...	100 per cent.
0·25 m. ,, ...	95 ,,
0·5 m. ,, ...	90 ,,
1 m. ,, ...	65 ,,
2 m. ,, ...	27 ,,
3 m. ,, ...	12 ,,
4 m. ,, ...	9 ,,
5 m. ,, ...	5 ,,
10 m. ,, ...	1·5 ,,

* 2 cm. below surface, as compared with air =77 per cent.

The water was evidently very turbid compared even with Poole and Atkins' Cawsand Bay results.

The above examples should serve to show what a very variable factor light intensity is in the sea.

Knudsen (1922, p. 14) gives figures for the coefficient of absorption of light of different wave-lengths in the Nyborg Fjord on June 11th, 1921. "The coefficient of absorption has a pronounced minimum for λ = 5100, *i.e.* in the green portion of the spectrum. From this point onwards the coefficients of absorption increase both towards the red and the violet end of the spectrum." The actual region of greatest penetration of the spectrum varies slightly from place to place according to the turbidity of the water, shifting towards the blue end as conditions become clearer.

Besides the visible spectrum, which stretches from about λ = 7000 (red) to λ = 4000 (violet), it is probable that the longer ultra-violet rays (λ = 4000 − 3000) penetrate to a certain distance. Grein (1914) demonstrated their presence by a photographic method down to 1500 m. in the Mediterranean. The deleterious effects of ultra-violet rays are well known (see p. 251), and further evidence of the presence of these rays, in the surface layers at any rate, is afforded by Fischer (1894) and Bertel (1912). They found that after midday the numbers of bacteria at the surface were much less than after midnight, the bacteria being killed off by the sun's light in the daytime and their population being enriched from deeper layers at night.

Lastly, light probably has a directional influence down to great depths. Helland-Hansen showed this to be the case in tropical waters at a depth of 500 m. (Murray and Hjort, 1912, p. 252).

Temperature, viscosity and density.

It may be said that as a general rule the temperature in the sea decreases with depth. The actual variations in temperature are of course different from place to place and from season to season.

In coastal waters, while in the winter the water layers are of the same temperature from surface to bottom, in summer, owing to the warming up of the surface layers, the temperature may decrease rapidly within a very short depth owing to the formation of a "discontinuity layer." The depth at which this layer occurs may be as much as 20 m. In the English Channel off Plymouth, for instance, on August 16th, 1921, the temperatures were (Harvey, 1923, p. 228):

Depth in m	Temperature in ° C.
5	16·02
10	16·02
15	15·77
20	13·68
25	13·59
30	13·51

In this case the discontinuity layer was at some depth between 15 and 20 m.

For the open ocean it is not possible to say more here than that the temperature decreases fairly rapidly for the first 50 or so metres, and then more slowly until great depths are reached where it remains more or less constant. There may in places be a further rapid decrease in the region of about 500 m. It is obvious also that in certain localities, such as the Baltic, there may be a very definite layering due to the surface and bottom waters being of different origins. Such cases cannot be regarded as usual.

In addition to the direct effect temperature can have on a marine organism there is an indirect method in which it can act. This is by altering the viscosity or frictional resistance of the water. The lower the temperature of the water the higher its viscosity, and hence the easier it is for animals and plants to float or maintain their level in the water.

I give below the changes that occur in the viscosity with different temperature conditions (Matthews, 1923, p. 671):

	Viscosity at	
	Salinity º/₀₀	
° C.	30	35
0	104·5	105·2
1	100·4	101·1
2	97·3	98·0
3	94·3	95·0
5	89·1	89·8
10	77·2	77·8
15	67·5	68·2
20	59·9	60·5
25	53·3	53·9
30	48·1	48·6

That the viscosity is felt by different organisms is abundantly evident when the structure of the minute floating life of warm-water regions is compared with

that of those from cold latitudes. The animals and plants from warm waters are much better equipped with spines and projections to act as organs of flotation (Ostwald).

The density of the surrounding water at times influences the vertical distribution of plankton organisms (p. 225). This, which is dependent on temperature, salinity and pressure, usually increases with depth. Generally, however, the changes are too slight to be of significance in the distribution of the plankton. It is only when a marked discontinuity layer is present and the density varies considerably within a short depth that a pronounced effect may be produced.

There are indications that in the sea there are periodic vertical oscillations of the different water layers (Helland-Hansen and Nansen, 1926)—see p. 241 of this paper.

Winds and currents.

Under certain conditions winds and currents may have considerable effects on the vertical distribution of plankton. For instance, offshore winds may cause water to upwell, in which case, if the water movement be rapid, plants and animals are carried up into the surface layers. Changes brought about in vertical distribution in this manner are purely mechanical results, and it cannot be said that the winds or currents are significant factors in the actual behaviour of the organisms, although the effects produced are commonly seen around coasts and in regions where one current meets another. For a good account of the effects produced by wind on the movements of water see Sandström (1918).

Pressure.

The pressure in the sea varies with depth. It can be stated roughly that this is 1 atmosphere for every 10 m. We thus see that if an organism passes through a vertical distance of as much as 50 m. in a short time it will experience considerable changes in pressure.

Regnard (1891, p. 154) subjected *Vorticella* and other Infusorians to pressures of as much as 600 atmospheres. He found that some of these organisms survived even this great pressure for 10 minutes. Others, however, could not withstand it and succumbed at 300 atmospheres. Some animals, such as Annelids and starfish, were still alive after being subjected to 1000 atmospheres. Fresh-water Crustacea, such as *Cyclops* and *Daphnia*, recovered after 5 minutes at 600 atmospheres, which is approximately equivalent to a depth of 6000 metres.

In the face of these experiments it would seem doubtful therefore whether pressure can be regarded as a factor of great importance in the vertical distribution of plankton.

Steuer (1912, p. 569) captured some of the Cladoceran *Evadne*, and the Copepod *Centropages violaceus*, from the surface and, enclosing them in a glass tube with silk tied over its ends, sunk them to a depth of 150 m. for a quarter of an hour. The animals were still living. He then sunk them to 300 m., and after half an hour, although the Copepods were still sound, the *Evadne* were all dead. On the assumption that these forms were highly eurythermal and euryhaline Steuer thought that the increase of pressure was the cause of death.

Salinity.

The salinity of sea water varies from region to region according to the degree of evaporation or the amount of dilution, whether by precipitation from the atmosphere or outflow of fresh water from the land. The concentration in open waters remains constant within very narrow limits, although it varies slightly with depth. For instance, in mid-Atlantic it may at first increase slightly with depth and then decrease. It is doubtful, however, whether such changes are of much importance in influencing the vertical distribution of plankton organisms. The changes that occur from depth to depth are, of course, much more marked in areas where water layers of different origin overlie one another; in such localities the effect on the vertical distribution of plankton is noticeable, either on account of the different densities in the case of the phytoplankton (see p. 223), or because the salinity at one level may be well outside the normal limits within which an animal lives (see p. 240).

Oxygen and CO_2 content, dissolved nutrient salts, etc.

The surface waters of the sea are usually saturated with oxygen, and this saturation is maintained both by mixing of the water layers and, at times, by the active photosynthesis of the floating algae. In deeper layers, below 100 m., in the open ocean, the oxygen content generally falls off owing to the respiration of the animal life. It is doubtful however whether it is ever low enough to affect the vertical distribution of plankton in open waters. In extreme cases, such as that of the Black Sea, absence of oxygen prohibits the life of many organisms. It is a remarkable fact, however, that Schmidt (1925) reports that in the Pacific near the Panama Canal, at a depth of 400–500 m., the water contained practically no oxygen, yet at 300 m., with an oxygen content of only about 2 per cent., the plankton life was even richer than at a corresponding depth in the Atlantic. Such low oxygen content can generally be regarded as indicative of stagnant conditions.

The CO_2 content of sea water again probably plays only a small part in controlling the behaviour of plankton organisms. It can generally be regarded as being at about the same pressure as in air, though this may be reduced by heavy flowering of Diatoms in the upper layers. Considering the effects obtained by Rose (see p. 250) it seems doubtful whether CO_2 changes are sufficient to produce much effect in open circulating waters.

I give here figures for the hydrogen-ion concentration of sea water at different depths (Palitzsch, 1911–13, p. 91):

Depth in m.	Atlantic west of Portugal pH	Mediterranean between Sardinia and Italy pH
0	8·22	8·23
100	8·13	8·21
400	8·04	8·19
1000	8·01	8·14
2000	7·95	8·09
3000	—	8·07

It can be seen that the changes are slight, and in view of the results obtained by Rose (see p. 250) with varying *p*H conditions on several plankton animals the importance of these changes is doubtful.

The presence or absence of dissolved nutrient salts, such as phosphates and nitrates, may play their part in controlling the vertical distribution of the phytoplankton (see p. 225).

Swimming and sinking speeds of different organisms.

In a study of the vertical distribution of plankton and the changes that occur in it, it is necessary to know at what speeds the different animals can swim upwards and also the passive sinking speeds of the plants and animals.

Groom and Loeb (1890, p. 174) give the speed of the nauplii larvae of *Balanus perforatus* as an average of 1 mm. a second at 15° C., *i.e.* 3–4 m. an hour.

Parker (1902) gives as the speed of ascent of the Copepod *Labidocera aestiva* 1 fathom (1·83 m.) in 18 minutes; and for its speed of descent 1 fathom in 6 minutes.

Steuer (1910, p. 389) gives for marine plankton crustacea the maximum current speeds against which they can hold their own; for an average-sized Copepod it was 80 seconds per metre, and for small decapod larvae 1 m. in 40 seconds.

Apstein (1910) gives the sinking speeds for a number of animals and plants in still water. For instance, the Diatom *Chaetoceras boreale* took on an average 4 hours 49 minutes to sink 1 metre in water of 35·31 ⁰/₀₀ salinity at 16·4° C.; *Sagitta bipunctata* took 3 minutes 17 seconds, and *Calanus finmarchicus* took 3 minutes 21 seconds to sink a metre, while *Pseudocalanus elongatus* took 10 minutes to sink the same distance. (See also Lohmann (1910, p. 291) for Appendicularians.)

THE VERTICAL DISTRIBUTION OF THE PHYTOPLANKTON.

The phytoplankton in the sea is composed mostly of Diatoms and, to a certain extent, of Green and Blue-green Algae, such as *Halosphaera viridis* and species of the genus *Trichodesmium* respectively. There is also another group of plankton organisms, the Dinoflagellates or Peridinians, which, on account of the undoubted powers of assimilation of many of them, are here included in the phytoplankton.

Our knowledge of the vertical distribution of this floating plant life has been gleaned chiefly from the results of large oceanographical expeditions, the most important of which from this point of view are the German Tiefsee *Valdivia* Expedition in 1898, the German *Deutschland* Expedition in 1911 and the cruises of the Norwegian research vessel *Michael Sars* in 1910.

The actual depths at which the phytoplankton is most abundant and at which it finds its limits for growth vary from place to place. In general it can be said that for open ocean waters the plant life is limited to the upper layers from the surface down to about 200 m., with a region of maximum abundance at about 40–60 m. (Fig. 1) (Karsten, 1905; Gran, in Murray and Hjort, 1912). The main factor controlling the depth distribution must be the intensity of light; this we know varies considerably from place to place and season to season; at the same time the penetration of the light will vary in different regions owing to greater or

lesser turbidity of the water. In coastal waters, where there is much matter in suspension, one would expect the region of greatest abundance of plant life to be at a lesser depth than in more offshore waters or under oceanic conditions. The results of collecting are quite in accord with this. Gran (1912, p. 126) concluded that in Northern European waters "the light optimum for far the greater part, if not for all, of our assimilating plankton algae is situated close to the surface, probably not as deep as 10 m., and we might perhaps even venture the assertion, that algae occurring in our latitudes with a maximum deeper than 30 m. have never any optimum of development down here."

Apstein (1906, p. 16), in the North Sea in May, found the surface layers from 0 to 5 m. richer than from 5 to 10 m.
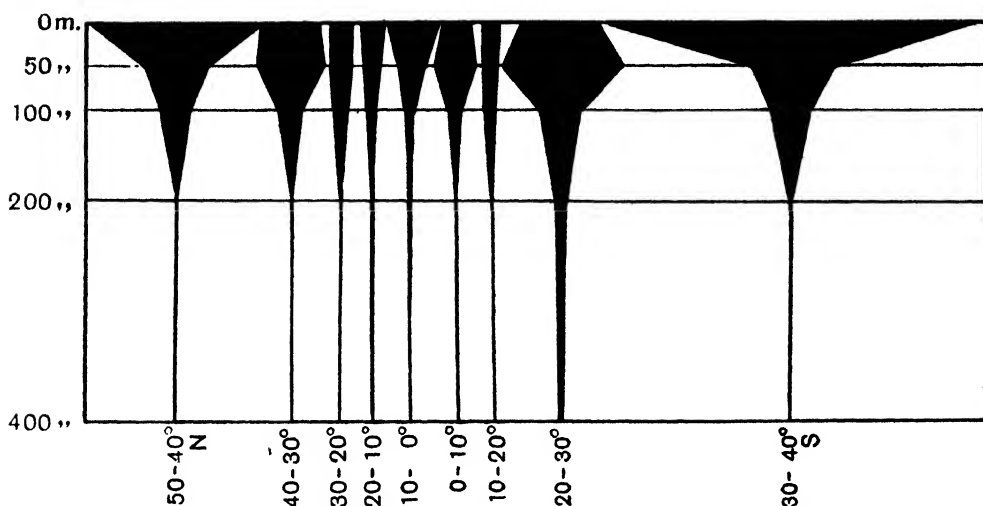
Fig. 1. Average depth distribution of Protophyta (with Chromatophores) in the Atlantic Ocean for each ten degrees of latitude. (After Lohmann, 1920, Fig. 74, p. 266.)

Lohmann (1902, p. 155), from a study of water samples off Syracuse in the Mediterranean, where the water is conspicuously clearer than our northern waters, found that there were more plant cells, especially Coccolithophoridae, at 50 m. than at the surface.

It was first noticed by Schimper, on the *Valdivia* Expedition, that certain species of Diatoms and Peridinians differed from others in their vertical distribution. He distinguished, in the Antarctic, "surface forms" (*Oberflächenplankton*) predominating down to about 60 m., and "deep-forms" (*Tiefenplankton*) or "shade flora" (*Schattenflora*) existing from the 60 m. level down to 200 m. He found 90 species of *Coscinodiscus* in this zone.

In the Indian Ocean and warmer parts of the Atlantic, where Peridinians—warm-water-living forms in general—predominated in the plankton, he found also surface-living forms existing mostly down to 60, 80 or 100 m., while below this occurred his "shade flora" stretching down to generally 150 or even 200 m.

Lohmann (1920, p. 278), in his comprehensive work on the centrifuge plankton of the *Deutschland* expedition, gives a complete series of forms whose regions of maximum abundance grade from the surface to 200 m.; no form had a maximum as deep as 400 m. His results gave that:

57 per cent. had a maximum at the surface

31	,,	,,	,,	50 m.
7	,,	,,	,,	100 m.
4	,,	,,	,,	200 m.

He further says that those species which have their optimum at 50 m. cannot be designated as "shade forms" (*Dämmerungsformen*) because almost always they are very frequent at the surface, and their centre may perhaps lie between 0 and 50 m. This term would, however, apply to those forms most abundant at 100 m., especially as some already clearly show in their form an adaptation to a region of low light intensity, *e.g.* the Diatoms, *Coscinodiscus* spp. and the Coccolithophore, *Deutschlandia anthos*. The species lastly which find their optimum conditions at 200 m. must be termed "deep forms" (*Tiefenformen*), *e.g. Coccolithophora fragilis*. He also concluded from his results that some species were adapted to a wide range of intensities and others to a narrow range.

In contrast to the normal vertical distribution of phytoplankton given above it is occasionally found that collections at sea give conflicting results. There are two chief causes for this.

1. *The natural weight of the plants* is such that they must tend to sink. Thus a sudden flowering of Diatoms in the surface layers will give rise to a large mass that slowly sinks into deeper water. These plants will eventually fall to a depth at which the light intensity is insufficient for photosynthesis; under such conditions in many species "auxospores" are being formed.

Gran (1912, p. 123) gives the following scheme for the distribution of Diatoms in northern waters in which the water layers are approximately homogeneous as regards temperature and salinity.

(*a*) When the maximum occurs at or near the surface, development is in an incipient stage.

(*b*) If the maximum be at 10–30 m., it will be due to the main mass sinking downwards but still in active production.

(*c*) If the maximum is at 50 m. or more, the main quantity of Diatoms will be on their way down towards deep water in a stagnating condition as regards assimilation and development.

An excellent example of the formation of resting spores in the deeper layers as the Diatoms are sinking down is furnished by Lohmann (1908, p. 249), whose figure I have reproduced here (Fig. 2). This represents the spring outburst of *Chaetoceras* spp. in the Baltic at Laboe in 1906. It shows well how, between April 4th and May 16th, the Diatoms gradually sank from the surface to 15 m. and how at the same time the numbers with spores increased in the deeper layers as the time advanced.

2. *Hydrographical conditions* may be at times a cause of irregular distribution in certain inshore waters and regions, such as the Baltic, where there may be two or more water layers of different origin overlying one another. In cases where
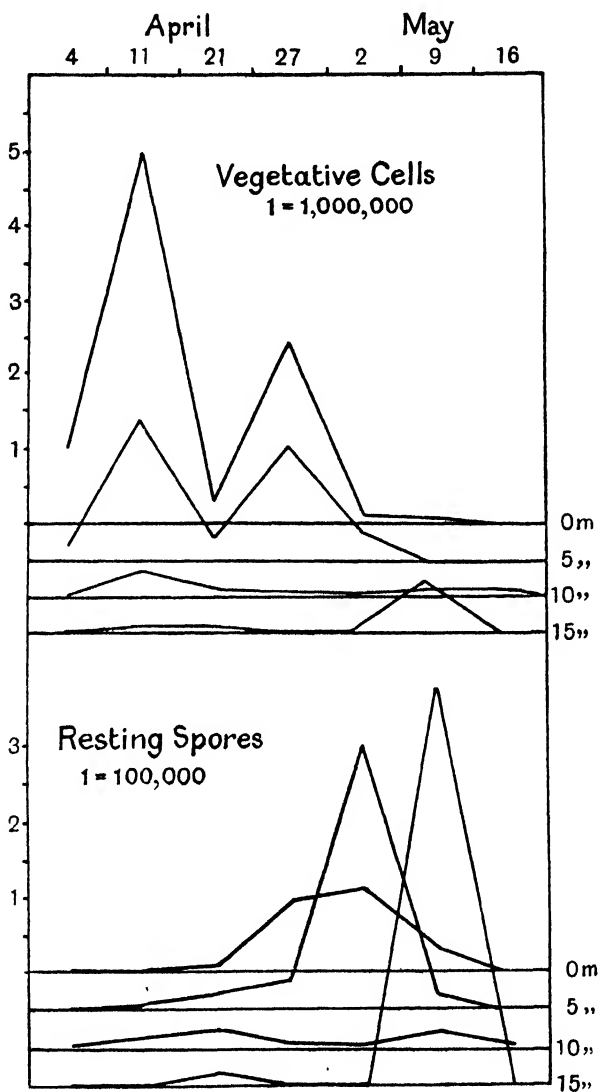
Fig. 2. Graphs showing the gradual sinking of Diatoms of the species *Chaetoceras* and the increase in numbers of cells containing resting spores. (After Lohmann, 1908, p. 249.)

there are marked discontinuity layers the mass of sinking Diatoms may be held up above the lower layer of dense water so that there is an abrupt passage at this depth from many Diatoms to extremely few. Occasionally heavy salt water carrying its characteristic flora may flow under lighter fresher water in which these plants

are absent. This does not necessarily imply that the plants in the lower layers are able to carry out active photosynthesis and grow at the depths at which they are found. A good example of this may be cited from Ostenfeld (1913, p. 436). In the Sea of Marmora the surface layers down to about 20 m. with a salinity of 22 $^0/_{00}$ carried with them a flora typical of the Black Sea, while at 25 m., and lower, where the salinity was 38 $^0/_{00}$ the forms were typical of the Mediterranean. In this case water was issuing from the Black Sea and spreading out as a surface layer over the Mediterranean water.

There is yet one further factor that may, under certain conditions, govern the vertical distribution. Sea water contains in solution nutrient salts such as phosphates and nitrates that are necessary for the growth of plant life. Atkins (1926, *a*) has recently shown that in the English Channel off Plymouth the phosphates may be almost entirely used up by the spring outburst of Diatoms. As the surface waters warm up in early summer a discontinuity layer is set up, and owing to the differing densities of the upper warm and lower cool layers mixing is practically inhibited except when there are very severe gales (Atkins, 1925). Should therefore the water above the discontinuity layer be denuded of phosphate, plants will be unable to grow at these levels and the reservoir of phosphate contained in the lower layers becomes cut off. Before the discontinuity layer has sunk below the depth at which the light intensity is too low for active assimilation it may occur that, while Diatoms are abundant in the deeper layers, they are much less numerous at levels above the discontinuity layer, those that were originally there having sunk to deeper levels. This has been shown to be the case in the Clyde Sea Area by Marshall and Orr (1927). Harvey (1926) has also found that the changes in quantity of nitrates in the sea run parallel with those of the phosphates.

Pavillard (1926, p. 68) remarks that in July and August the Tyrrhenian and Ionian Seas are deserted by Diatoms in the surface layers, and yet in the Straits of Messina an astonishing fertility prevails. This is presumably due to the upwelling of phosphate-rich waters in the Straits.

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Experimental evidence.

Regnard (1891, p. 231) experimented by growing cress and radishes in glass vessels sunk at different depths in the sea off Monaco. He found that it was only when one reached a depth of 30 m. that the light was so low in intensity as to give rise to only a poor development of chlorophyll; it should be stated that the sea in this locality is very clear. Whipple (1908, p. 102) immersed fresh-water Diatoms in bottles at various depths in a lake. He noted that growth at a depth of 6 in. (15·2 cm.) was greater than that actually at the surface, where sunlight was apparently too strong. The depth limit for growth varied according to the colour and transparency of the water, *e.g.*:

Colour	86	Limit	8 feet (2·4 m.)
,,	60	,,	12 ,, (3·7 ,,)
,,	29	,,	15 ,, (4·6 ,,)

He gives a figure showing the remarkable agreement between the growth of Diatoms and the intensity of the light at various depths. These very small depth limits, compared with those given above in the results of field investigations in the sea, are due to the much greater turbidity of fresh water in comparison with sea water and hence the much smaller penetration of light.

It was noticed by Schimper (Karsten, 1905, p. 13) that in certain Diatoms found near the surface of the sea a phenomenon described as "systrophe" occurred, *i.e.* the chromatophores were collected together in a clump in the centre of the cell, while in the deeper layers the chromatophores tended to be evenly distributed throughout the cell in a condition known as "peristrophe." Schimper experimented by placing different species of Diatoms in vessels exposed to full daylight, shade and darkness. Although performed under somewhat unsatisfactory conditions, the results tended to show that surface forms such as *Chaetoceras, Rhizosolenia* and *Thalassiothrix,* were adapted to high light intensities and were extremely sensitive to the lack of light, which in a very short time brought about death. But species such as *Coscinodiscus,* which normally lived in the deeper layers, were relatively insensitive to complete darkness and among these *Actinocyclus* survived the longest.

Gail (1918, p. 149) found that no Diatoms grew below 6 m. on shells suspended at different depths in Puget Sound. (For culture experiments in the sea *vide* Gaarder and Gran, 1927.)

Owing to the selective absorption of sea water it is necessary to know which wave-length is most effective for photosynthesis. It has long been known that red light has the maximum effect on green plants, while the blue plays a less important part (Strasburger, 1912, p. 215; Regnard, 1891, p. 227, Kniep and Minder, 1912, and recently Klugh, 1926, *a*).

Miquel (1892, p. 343) asserts that for fresh-water Diatoms the yellow rays are most efficient and after them the blue and the green. Allen and Nelson (1910, p. 454), however, say that satisfactory cultures could not, in their experience, be obtained under conditions of yellow light with their total intensities.

Lohmann (1920, Table 156) has shown by a study of the structure of Coccolithophoridae from different depths a correlation between the development of their skeletal plates and the light intensity.

THE VERTICAL DISTRIBUTION OF THE ZOOPLANKTON.

It was first definitely shown by the *Challenger* Expedition that the water layers below the actual surface are inhabited by animal life down to depths of as much as 2000 fathoms (3658 m.), and it was stated that results "produced a strong belief that all the intermediate zones of life were inhabited." These conclusions were amply confirmed by the results of the *Valdivia* Expedition in which closing nets were employed to prevent the capture of organisms as the net was being hauled to the surface. Hjort remarks of this expedition that: "Even in hauls between 5000 and 4000 m. living crustacea as well as larvae of the same animals were captured—a sufficient proof that these animals not only live but also breed at these depths" (Murray and Hjort, 1912, p. 562).

Animal life, however, is not distributed evenly from the surface to the bottom; there is a zone, generally a few metres below the surface in inshore waters, in which animals are most abundant: in the open ocean this may be as deep as 500 m. or more for Copepods, especially where a "rise in the specific gravity and viscosity occurs" (Murray and Hjort, 1912, p. 723). At the same time different species each appear to have their own definite vertical regions of distribution with upper and lower depth limits: this is only to be expected on account of the different environmental changes which occur from the surface downwards, as outlined above. The occurrence of these changing communities to be found at different depths has given rise to many attempts to classify the organisms according to their vertical distribution, and various schemes have been put forward for dividing the depths into community zones or zones based on external physical factors. As Hjort (Murray and Hjort, 1912, p. 562) has pointed out, however, "such distinctions are arbitrary, because our knowledge of the bathymetrical distribution of animals is limited, because the laws of distribution are imperfectly understood (for instance, the effects of light), and because the bathymetrical occurrence of certain species is subject to great variation in different regions." For this reason Hjort uses only the word "bathypelagic" to denote those animals that live deep in the intermediate layers.

I have given here (p. 228) in tabulated form the most important schemes for vertical distribution that have been suggested. It must be understood, however, that in no case can the depths given be regarded as hard and fast, but rather that one zone merges into another: this was fully realised by the authors of the various schemes.

Damas and Koefoed (1907, p. 421) have regarded Lo Bianco's Phao-, Knepho-, Skoto- and Panteplankton, as the best guide to depth distribution, if applied not to zones but to individual species. The advantage of this classification is that one is not tied down to actual depths, because we know that the strength of light and its penetration varies from time to time and place to place, and the scheme implies the formation of zones that are constantly fluctuating in depth. It must of course be understood that these divisions are only of the very broadest; it is unlikely that one would find a community of organisms, for instance, to which the title "Phao-plankton" could be assigned to distinguish it from the community below. It is most probable that the vertical distributions of the different species gradually merge together to form a community of ever-changing composition from Šurface to bottom. A good indication of such changes has been shown for inshore waters, Fig. 3 (Russell, 1926, *c*, p. 416). It has been noticed that the actual range of depth
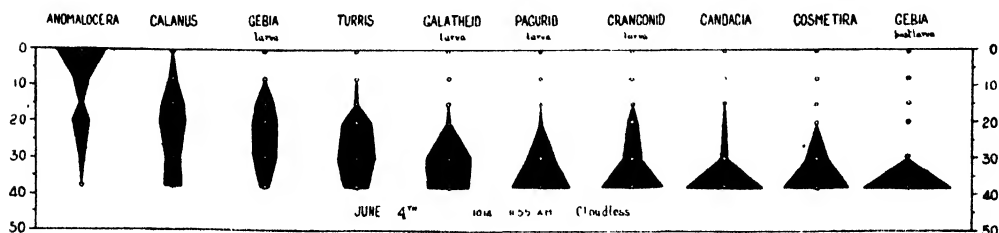
Fig. 3. The vertical distribution of ten species of plankton animals on the same day showing the gradual transition from those with their region of maximum abundance at the surface to the deep living forms. The depths are in metres. (After Russell, 1926, *c*, p. 416.)

Some Suggested Schemes for Classification of Vertical Distributions.

Fathoms	Metres	Dahl 1894 Copepods	Haeckel 1887 Radiolaria	Fowler 1898 Zooplankton	Lo Bianco 1903 All plankton	Karsten 1907 Phytoplankton	Haecker 1908 Radiolaria
Surface							
25	30 50 60	Surface region (Oberflächen-region)	Pelagic	Epiplankton	Phaoplankton	Increase of plankton or	Colliden-schicht
50	100		Pellucid			or Shadow or Twilight Flora	or
	150				Knepho-plankton		
100	200					Decrease of plankton	Challenger-idenschicht
150	300						
200	400		Obscure	Mesoplankton		Plants as good as absent	Tuscaroren-schicht
	500	Middle region (Mittlere-region)					*a* - 400–1000 m. Pandora-stufe
1300					Skoto-plankton		*b* - 1000–1500 m. Aulospathis-stufe
400							
			2000 f. Siliceous				
1000		Deep region (Tiefen-region)	3000 f. Abyssal	100 f. above bottom Hypo-plankton			1500 m. Pharyngellen-schicht 5000 m

distribution varies in size for different species, *e.g.* the Copepod *Calanus finmarchicus* which in inshore waters exhibits a distribution extending through wide limits, and the Medusa *Cosmetira pilosella* whose range is very confined (Russell, 1926, *c*). For those forms with a very extended range such terms as "Eurybathic" (Dahl) and "Panteplankton" (Lo Bianco) have been suggested.

It is of interest to record here cases in which the vertical distribution of plankton animals is apparently limited by the presence of symbiotic Algae within their tissues. Among Protozoa it is well known that many Radiolarians carry within them Zooxanthellae, and these forms are only found in the upper water layer (see below). Conklin (1908, *b*) records the presence of symbiotic Algae resembling Zooxanthella in two actinian larvae, *Zoanthella* and *Zoanthina*. He says: "The fact that these larvae are found at the surface at a period of the day when most pelagic larvae have settled to deeper and darker levels may be associated with the metabolism of this symbiotic alga." Uchida (1926, p. 52) notes that the ephyrae of a rhizostome Medusa, *Mastigias papua*, could be caught at midday at the surface and not at night. He says: "This is related to the presence of the yellow cells which are embedded in the jelly." The adults also possess these symbiotic algae and are abundant on the surface at noon time on sunny days, though "when it rains or blows hard, they descend to a greater depth."

In considering the vertical distribution of zooplankton as a whole it is necessary that we should treat the minute organisms and the larger animals separately. It is evident that power of locomotion must be an important factor in the distributions shown and such changes as may occur in them. Accordingly I will deal first briefly with the Protozoa, and then with the Metazoa around whose behaviour most of the discussion and theories regarding vertical distribution have centred.

PROTOZOA.

For want of space it is only possible to make short mention of the vertical distribution of the Protozoa. One of the most important groups in the plankton is the Radiolaria.

Haecker (1908, p. 553) says that in the uppermost layers from o to 50 m. (Lo Bianco's Phaoplankton zone) the Radiolaria with Zooxanthellae make up a large part of the microplankton; these consist of Spumellaria, Nasellaria and Acantharia, but only very few of the Tripylaria which do not possess the commensal Algae. In the layers below this and in the depths between 400 and 1000 or 1500 m. are most of the Tripylaria. A study of Haecker's summary on the vertical distribution (1908, pp. 554-5) shows that in common with other plankton animals each species has its own depth zone.

Lohmann (1920, p. 256) gives the region of greatest abundance for the Protozoa consisting of Acanthometrids, Globigerinas and naked Flagellates and Ciliates, as between 100 m. and the surface although many occur between 100 and 200 m.

Gamble (1909, p. 96) quotes Brandt, who says that *Thalassicolla* controls its vertical distribution in the water by the formation and expulsion of vacuolar fluid.

In rough weather they sink. Haeckel (1887, p. clii) says that in rough weather surface forms sink to 20 or 30 fathoms. (See also Schewiakoff, 1926.)

METAZOA.

All the chief groups of the Invertebrates are represented in the plankton; at the same time the young of most fish and the adults of many small oceanic species can be included. With few exceptions, such as the Siphonophores *Velella* and *Physalia*, which are blown by the wind, these animals are capable of individual movement. Although in most cases their power of locomotion is insufficient to allow significant movement in horizontal directions compared with the movements of the water masses within which the animals occur, it is nevertheless by their movement, aided by floating devices, that the animals are able to maintain their vertical distribution and bring about such changes as may occur in it. A few species, such as the larger Euphausiids, are probably capable of considerable migrations in a horizontal direction, lying as they do on the borderland between the arbitrary distinctions of plankton and pelagic organisms.

It has been noted above that the observed distribution for many animals shows an orderly arrangement and that it is not simply haphazard; that while some species live mostly at one depth, others may live at another level. This has been shown to hold for all groups, and the data can only be obtained by perusal of the reports of the different groups obtained on the various oceanographical expeditions. At the same time it has been shown for any one species that the vertical distribution exhibited is not to be laid down within fixed limits but is liable to variations, the most important of which can be included under the following headings:

1. Regional changes in vertical distribution.
2. Seasonal changes.
3. Daily changes.
4. Ontogenetic changes.
5. Changes due to spawning habits.
6. Special variations due to hydrographical conditions.

It will be convenient to deal with each of these in turn. So far the possible correlation between the vertical distribution of different species and the external factors of their environment has not been discussed. It is, however, an examination of the changes that occur in the distribution of the animals that has naturally been the most fruitful as a point of vantage from which to elucidate the problem of vertical distribution as a whole, just as when studying the causes of animal behaviour in the laboratory it is necessary to vary the causative factors in order to note the resulting reactions. Changes in vertical distribution in nature are most probably mainly connected with alterations in environmental conditions. In the following paragraphs therefore the various explanations that may have been put forward by the different observers are briefly stated.

Regional changes in vertical distribution.

It has been noticed that in one locality a species may be most abundant at certain depths while in other regions the centre of maximum abundance will be situated at a different level.

Damas and Koefoed (1907), in a study of the distribution of Copepods in the Greenland Sea, observed changes in depth distribution according to region. They found that species of the intermediate and deep layers (Knepho- and Skotoplankton) were met with in the ice-covered western part of the Greenland Sea at much higher levels than in the eastern uncovered portion. They further, on an examination of the hydrographical data, concluded that this difference was not occasioned by currents and had no relation with salinity or temperature. The further south also that they went the deeper the same species were below the surface.

They say (1907, p. 421): "The fact that a number of deep species (Skotoplanktonic) rise in polar regions to the immediate neighbourhood of the surface finds a natural explanation. Nansen has shown, in fact, that light does not absolutely penetrate through the ice"; and on p. 421: "Notre étude nous amène à admettre que le niveau choisi par la même espèce sous différentes latitudes est réglé par la quantité de lumière."

They sum up as follows: "At a given place the organisms are distributed at different levels according to the degree of light for which they are sensitive. They rise and fall according to the daily variations in light intensity. They move equally in a vertical direction from season to season. The level at which one form keeps is different from sea to sea. Species which live at the surface at the pole are found in the depths under the equator. Others that are seen to exist in the intermediate layers in the north are only observed in the south in the abyss. Then, the zones of distribution are, not horizontal, but oblique."

The vertical distribution of the Chaetognatha has been studied especially by Fowler (1905, 1906, 1907), Ritter Záhony (1909, 1910), Michael (1911) and Huntsman (1919). A similar change in vertical distribution from place to place has also been observed for these animals. The most striking example is to be found in the species *Eukrohnia hamata*, a bipolar species which lives at great depths in tropical and subtropical regions of the ocean and gradually rises nearer the surface towards the two poles. Both Fowler and Ritter Záhony appear to have regarded temperature as the factor of importance that causes this regional change in vertical distribution. This factor was also regarded by Chun (1897) as important when he put forward his theory of bipolarity. These changes are explained by the fact that the northern species do not tolerate water above a certain temperature, and in sub-tropical and tropical regions, in order to avoid the warm surface layers, they seek the greater depths where their usual low temperatures are to be found.

Browne (1910, p. 10) similarly notes the possible significance of temperature in the distribution of Medusae. He says: "The occurrence of *Periphylla* at the surface in the Antarctic tends to show that the 'deep sea' medusae are lovers of cold water, or at all events, flourish best at a cool temperature. If the temperature of

the water fixes their bathymetrical distribution, then we can account for their keeping below the warm water zones in the tropical and temperate regions."

Bigelow (1909, p. 234) considered for the Medusae living at "intermediate" depths that "it is probable that it is not the increase in temperature, but light, which is the more important factor of the two in limiting dispersal."

Hjort (Murray and Hjort, Ch. x, 1912) gives some exceedingly interesting observations on the vertical distribution of pelagic and planktonic animals. In a discussion on the colours of marine animals at different depths (p. 665) he says: "We have seen that the upper limit for *Cyclothone microdon* and the red crustaceans, in the Northern section from Newfoundland to Ireland, or about lat. 50° N., was approximately 500 m. below the surface, and we have also noticed that the limit of depth for the same forms at the southernmost stations, or about lat. 33° N., was some 200–300 m. deeper." In previous observations in the Norwegian Sea, in about lat. 67° N., he found pelagic red prawns at depths of about 200 m. below the surface. By allowing for the altitude of the sun and transparency of the water at these three latitudes he finds that "there will be the same *intensity* from the rectilinear rays,

In lat. 33° N. at about 800 m.
    ,,   50° N.   ,,   500 m.
    ,,   67° N.   ,,   200 m.

The red and black animal forms, therefore, as has been found in the investigations I have just described, have an upper limit in the different waters which corresponds everywhere with the same intensity of light."

Esterly (1912, p. 334) says: "It may be pointed out, however, that the upper limit for red animals as given by Hjort for this latitude (700–800 m. or about 435 fathoms) seems to be farther down than our data would lead us to believe. The extreme limits within which the largest numbers of individuals are taken are 200 and 400 fathoms, but horizontal closing hauls below 350 fathoms are greatly needed to determine this point more satisfactorily."

Unfortunately there are as yet no sufficiently accurate observations on vertical distribution in the open ocean correlated with light intensity measurements. In the writer's opinion, however, the above point, viz. that it is necessary to go 600 m. deeper in lat. 33° N. to find the same light intensity as at 200 m. in lat. 67° N., is of very great significance and points possibly to the prior importance of light over temperature as a determining factor in these regional changes of depth distribution.

Seasonal changes in vertical distribution.

The phenomenon known as "seasonal migration," *i.e.* change of level from time to time in the year of a single species, has long been known. It was first brought into prominence by Chun (1888) who referred to records by Schmidtlein (1879, 1881), and by Lo Bianco, which showed clearly the times of appearance and disappearance of certain species in the surface layers in the Mediterranean. It was observed that some species that occurred at or near the surface in the winter months were not present there in the summer, and Chun by employing closing nets demon-

strated without doubt that their disappearance in the summer was due to their retreating to the deeper levels. He considered that the heating up of the surface waters in summer was the factor mainly responsible for the departure of the surface forms to deeper layers where the temperature of the water was considerably lower.

In recent years these observations have received ample confirmation. For instance, Stephensen (1926), on examination of the collections of hyperiid Amphipods made by the Danish research vessel *Thor* in the Mediterranean, found that *Phronima sedentaria* was captured in the daytime in winter with 65 m. of wire out or deeper, while in daylight in the summer they were only to be taken with 300 m. of wire or more.

Similar observations were made for fish captured on the same expedition. Jespersen and Vedel Täning (1926) found that the adult and adolescent stages of *Cyclothone Braueri* had their upper limit in winter "at only about 100 m., as against about 500 m. in summer."

For coastal waters it has recently been shown (Russell, 1926, *c*, and Bigelow, 1926, p. 202) that the Copepod *Calanus finmarchicus* apparently exhibits seasonal variation in its vertical distribution between the months of April and August in the daytime. Evidence is given by Russell supporting the theory that light intensity is the most important factor; the region of maximum abundance of *Calanus* was at its deepest in mid-June when the light intensity was greatest, whereas these Copepods were high in the water on a foggy day in August when the surface layers had reached their maximum temperature for the year.

Daily changes in vertical distribution.

The so-called vertical migrations or wanderings of plankton animals at night have long excited the interest of zoologists. It is now fairly established that such indeed do occur. Doubt has been thrown on the truth of such a phenomenon by Franz (1910), who suggested the possibility that the organisms could see the net in the surface layers in the daytime and evade capture. To a certain extent he is justified in his arguments, especially when very small nets are employed. Robert (1922), working in fresh water, showed that the faster a small net is towed the more crustaceans it captures and considered this a good indication that the animals could avoid the net at slow speeds. Southern and Gardiner (1926, p. 144) came to a similar conclusion. Swiftly moving animals can also evade capture, but they do not strictly come under the category of plankton.

The employment of nets with mouths of a sufficiently large diameter to prevent escape has definitely shown that many plankton organisms move upwards towards the surface from deeper levels after or about sunset, returning once more to the deep water at the approach of daylight.

A close study of these vertical migrations in the sea has only been undertaken by a few observers. Most of the results of expeditions give only very slight information, the number of organisms captured being generally too low to be significant and the depths at which hauls were made being too wide apart to show detailed behaviour. Fowler's results obtained in the Bay of Biscay on the *Research*,

in 1900, produced a certain amount of information, but the two limitations mentioned above may be said to apply to these also. Hjort (Murray and Hjort, 1912) gives much general information, as do most reports on the special groups collected by other expeditions, but beyond showing that vertical migrations do occur in certain species it cannot be said that they produce sufficient detailed evidence to throw light on to the causes of the migrations.

Michael (1911) contributed a large amount of knowledge on the vertical movements of certain Chaetognatha from collections made at different depths in the San Diego region, California. Although the methods of the research, as admitted by Michael, cannot be said to be ideal, because nets of different fishing capacities were employed and the numbers in all catches over a long period for the different times of day were lumped together so that weather conditions were not taken into account, the results do, however, appear to produce very definite indications. Michael gives observations for several species, but I will only summarise here his results for *Sagitta bipunctata*, the species for which he has the most data.

Briefly his findings may be stated thus:

4–6 a.m.	they are most abundant at	7–12 fms.	(12·8– 22 m.)
8–10 a.m.	,,	,,	15–20 ,,	(27·4– 36·5 m.)
10 a.m.–12 n.	,,	,,	40–75 ,,	(73 –137 m.)
12 n.–2 p.m.	,,	,,	25–35 ,,	(45·7– 64 m.)
6–8 p.m.	,,	,,	surface and 4–6 fms. (7·3 -10·9 m.)

Thus we see that there is a gradual descent from near the surface in the early morning reaching a maximum depth at midday, and then a gradual ascent until they arrive at the surface about 8 p.m. There seems to be a slight indication that the *Sagitta* leave the surface during the night to return there once more before dawn.

Michael also made careful comparisons of the occurrence of *Sagitta* at the surface and the surface salinities and temperatures. He found quite a marked correlation between frequency of occurrence at the surface and the variations in the temperature, the animals remaining on the surface in greatest numbers when the temperature of the water was between 15·9° C. and 17·5° C. They were less frequent at the surface at higher temperatures, *e.g.* the frequency of occurrence at the surface between 10 a.m. and 2 p.m. was

15·9°–17·5°	...	...	...	83
17·6°–19·5°	...	...	...	67
19·6°–21·5°	...	...	...	33

At the same time he found a slight correlation with the salinity changes, the occurrence of *Sagitta* at the surface being most marked when the salinity lay between 33·605 ⁰/₀₀ and 33·648 ⁰/₀₀.

As a result of these observations his conclusions are as follows (Michael, 1911, p. 144):

"Notwithstanding that these more accurate methods of collecting were not employed, from what data we have the following conclusions may be drawn:

"1. The region of 15–20 fathoms is the center from which the species migrates

or, in other words, it is the depth in which the greatest number of optimum conditions favorable to this species are found.

"2. It migrates to the surface at sundown and sunrise because the conditions of light intensity at these times are *similar* to those occurring in 15–20 fathoms during the greater part of the morning and afternoon.

"3. Other things equal, it remains on the surface in greater numbers when the temperature of the water is between 15·9° and 17·5°, because this is approximately the normal temperature occurring in 15–20 fathoms.

"4. It remains on the surface in greater numbers when the salinity of the water is between 33·605 and 33·648, because this approximates the normal salinity in 15–20 fathoms. This, at present, is *barely suggested* by our data; it still remains to be proven.

"5. The animals leave the surface at night because the light incentive which caused them to migrate upward is absent, and they presumably return to the region of 15–20 fathoms where optimum temperature and other conditions occur.

"6. Probably light has more pronounced effect on vertical distribution than temperature or salinity, because its variations are more regular and periodic.

"7. All individuals do not react toward light, temperature, and salinity in the same way. While the majority migrate toward the surface during twilight hours, and toward deeper water during intense light and darkness, *a few almost always remain in deeper water during twilight, and on the surface during intense light and darkness*. Similar individual differences occur with respect to temperature and salinity. This means that those optimum conditions favorable to the species as a whole are not favorable to each individual or, in other words, the characteristic organization, constitution, or physiological state of each individual modifies the effect of light, temperature, and salinity on its behaviour."

A similar behaviour has been noted by Oberwimmer (1898, p. 574) for Heteropods and Pteropods, viz. movement to surface at dusk, sinking at night, and a smaller rise before sunrise.

Esterly (1912) has investigated the vertical distribution of 19 species of Copepods in the San Diego region, and for 16 of these his data show definite indications that vertical migrations occurred.

I give here his observations on *Calanus finmarchicus*, the most abundant form.

Number of Calanus *caught per hour at surface.*

Time			Time		
6–8 a.m.	...	91	6–8 p.m.	...	175
8–10 a.m.	...	80	8–10 p.m.	...	973
10 a.m.–12 n. ...	...	10	10 p.m.–12 m. ...	...	1375
12 n.–2 p.m. ...	...	10	12 m.–2 a.m. ...	...	58
2–4 p.m.	...	68·5	2–4 a.m.	...	97·2
4–6 p.m.	...	69·2	4–6 a.m.	...	19·3

These figures give abundant evidence of the vertical movement undertaken by *Calanus* and show that the maximum numbers taken at the surface occurred before midnight.

Esterly attempted to correlate the distribution of *Calanus* with salinity changes and concluded (p. 294): "On the whole, we are not warranted at present in forming a definite conclusion as to the effect of salinity on distribution." At the same time he thinks it doubtful if such correlation will be found, because the salinities occurring in that locality lie well within the limits of 35·30 ⁰/₀₀ and 33·00 ⁰/₀₀ that Farran (1910) gives for the range of salinities within which *Calanus* normally lives.

He also says (1912, p. 292): "The data concerning distribution and temperatures are given for what they are worth; they do not seem to show that temperature is important in determining the distribution of *Calanus* at the surface. There is doubtless a temperature that may be called usual at the region of maximum abundance during the day, but there is no evidence that *Calanus* seeks that depth because of the temperature. In view of the movements performed by the species, it is difficult to imagine the part that temperature really has in the life of these animals. The bulk of the population periodically leaves the region where the temperature is about 9 degrees on the average and moves into water where the average temperature is about 17 degrees. The temperature does not vary periodically or constantly enough to lead us to consider its changes as the causes of the migrations, and it is very probable that there is some other cause. The variations in the intensity of light are both constant and periodical, and it is my belief that light is the primary cause of the movements of this species and also the main factor in determining its vertical distribution."

Both Michael and Esterly emphasise the desirability for more precise data and give it to be understood that the views put forward by them are, at most, tentative, although the indications may be strongly in favour.

Recently Russell (1925) has carried out observations in shallow waters. The information obtained showed the behaviour of about 50 different species of animals throughout one night in July, in the English Channel off Plymouth. It indicated that for many forms there was a definite movement towards the surface, some species appearing to migrate right to the surface with a marked diminution in their numbers in the deeper layers, others merely filling up the surface layers so that their distribution seemed to be more or less uniform from the surface downwards, and yet others, which normally live on or very near the bottom in the daytime rising to about 20 m. above the sea floor.

It seems most probable that the types of distribution shown by the different species are due to a combination of the speed of upward movement of which they are capable and the time at their disposal for such upward migration (see p. 238). On the night in question there was a full moon, and an observation carried out on a dark night on the following year (not yet published) showed that many species moved higher in the water than when there was a full moon: the observations are however obviously too few to conclude whether the moon may have an influence. It was further noteworthy that on the moonlight night many forms appear to have shown little or no upward movement.

Other recent observations on the vertical migrations of plankton organisms have been obtained by Savage (1926) on general plankton, and by Hickling (1925),

who showed in a very striking manner that certain Euphausiids, *e.g. Meganyctiphanes norvegica*, left the sea floor at night and that their disappearance and reappearance accorded well with variations in light intensity throughout the 24 hours. Johansen (1925) and Russell (1926, *b*) gave observations on the diurnal movements of young fish.

It will be convenient here to review the situation as regards the causes of these daily changes in vertical distribution.

The suggestion put forward by Michael was that *Sagitta bipunctata* has an optimum intensity at which it lives. Thus as the light decreases towards night the animal must follow this intensity towards the surface. During the night the intensity falls far below the optimum and, the stimulus for upward movement
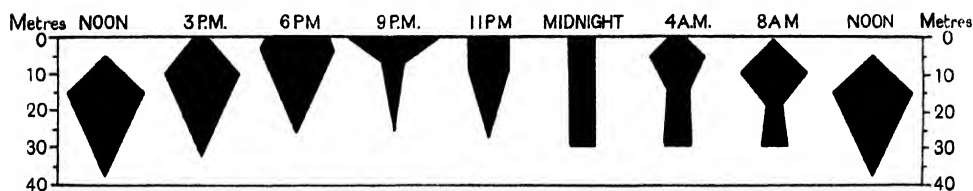
Fig. 4. Hypothetical vertical distributions at different times in the twenty-four hours to illustrate the suggested behaviour of a population of a species such as *Calanus finmarchicus*, in its diurnal movements when sunset occurs at about 8 p.m. based on actual observations (Russell, 1925, p. 791).
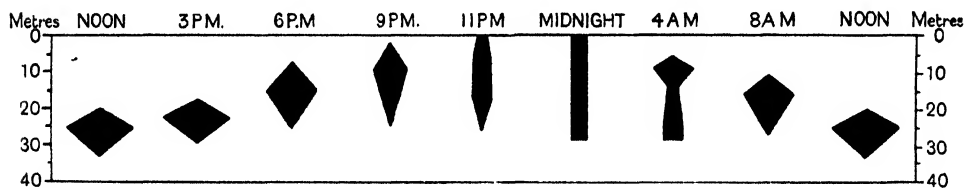
Fig. 5. Hypothetical vertical distributions at different times in the twenty-four hours to illustrate the suggested behaviour of a population of a species such as *Cosmetira pilosella*, in its diurnal movements when sunset occurs at about 8 p.m. based on actual observations (Russell, 1926, *c*, p. 432).

being thus removed, the *Sagitta* returns to the deeper layers where it finds optimal temperature and salinity conditions. As the light increases in strength the following morning it is once more attracted to its optimum intensity near the surface and this it follows downwards as the daylight grows.

Strong indications in favour of this theory are given by Russell's results. An examination of many of the distribution diagrams obtained shows that there is a movement towards the surface as the light fails, but that at night when the lowest intensity has faded away and directional light stimulus is removed, the animals become free to go where they will and become evenly distributed from top to bottom. As the light increases the following day those near the surface mass around the optimum intensity as soon as it arrives and follow it downwards, ever increasing their numbers by recruits from deeper layers who gradually come into the sphere of influence of the light or have been prevented from reaching the optimum on

account of slow movement. I give here diagrams to illustrate this behaviour (Figs. 4 and 5). Fig. 4 shows what might be expected of a species such as *Calanus finmarchicus* which lives, or rather has its region of maximum abundance, in the upper well-illumined layers at about 15 m. in the Plymouth region in the daytime. These diagrams have been based on the actual distributions obtained for this species on July 15th, 1924 (Russell, 1925). In Fig. 5 is given diagrammatically the behaviour to be expected of a species such as the Medusa *Cosmetira pilosella*, which in the same locality normally is most abundant in the daytime at about 25 m., where it must experience a comparatively low intensity of light. The even distribution at night and apparent picking up of an optimum intensity at dawn is beautifully shown by the actual results of two nights' collecting (Russell, 1926, *c*, p. 432), on which this diagram is based. It is probable that, owing to the sudden fading of light in the evening, this species never has time to reach the surface *en masse*, but the stimulus that brings them towards the surface is removed when they are about half-way up and they then assume an even distribution throughout the water layers. The combination of this very rapid fading of the light at sunset and the different speeds with which the various species can swim upwards will probably account for the fact that some animals appear to show a massing of their whole population at the surface, while others can only get a certain distance and then become uniformly distributed. We should therefore expect a greater congregation at the surface on an evening following a very dull afternoon on which, owing to the low light intensity, many animals will already be high in the water.

Michael, as noted above (p. 235), suggests that at midnight the animals (*Sagitta*) return to deeper layers and mass around an optimum salinity and temperature. He bases his suggestion on the fact that less *Sagitta* were found on the surface when the temperature was high than when it was low, and therefore that colder temperatures are more favourable and the animal can find these conditions in the deeper layers.

It seems to the writer that this can be more simply explained by the fact that sensitivity to light increases with rising temperature, owing to accelerated photochemical reactions, and that therefore in the daylight there are fewer on the surface when it is warm than when it is cold; the optimum intensity may also be slightly lower under warm conditions. Michael, however, also has indications that there are fewer on the surface at night when the water layers are warm, the light being then absent. This may be explained by suggesting that owing to increased sensitivity as the animals pass through the warmer layers they have been slightly retarded in their upward migration and less had reached the surface before the light stimulus had been removed.

It must be realised that these suggestions would apply only under ideal conditions, other factors, such as the occurrence of food, might hasten or delay the movements of individuals. In nature, too, a community of one species is generally heterogeneous, being composed of individuals of different ages whose reactions are not quite the same (see p. 239). At the same time they may refer only to certain species and cannot as yet be regarded as generalisations for all species.

Ontogenetic changes in vertical distribution.

A species may be found to occur most abundantly at a certain depth when adult, but while young it may have a different vertical distribution. The gradual changes in vertical distribution throughout the life of an individual have been termed "ontogenetic migrations." This has been noticed to take place in the case of very many plankton animals, in fact it is reasonable to suppose that it is a general rule.

In some cases it is found that the younger stages occur higher in the water than the adults; in others, and this is the least general, the reverse is the case. It will be sufficient here to cite only a few examples.

Fowler (1905, p. 76) gives the average lengths for the Chaetognath *Krohnia hamata* at different depths:

	50 fathoms	..	...	...	10	mm.
	75 ,,	...	...	...	10·3	,,
	100 ,,	...	...	...	10·5	,,
	150 ,,	...	...	...	11·9	,,
	200 ,,	...	...	...	11·4	,,
	250 ,,	...	...	...	13·9	,,
	300 ,,	...	...	...	13·6	,,
	350 ,,	...	...	...	12·7	,, ·
Closing net,	750– 500 fathoms		...	...	18·8	,,
	1000 750 ,,		...	...	21·3	,,
	2000 1000 ,,		...	...	20·0	,,

Here we see a gradual increase in length with depth.

Farran (1910) and Kraefft (1910) have noticed the same for Copepods. Santucci (1925) shows it for *Squilla* larvae   to mention only a few.

Jespersen and Vedel Tåning (1926) note it for the small oceanic fish, the Sternoptychidae. They say of *Vinciguerria atenuata*: "We find that the smallest postlarval stages are proportionally more numerous at depths fished with 25 and 65 m.w., and that the postlarvae, as they approach metamorphosis, tend to move down into deeper water (cf. *Cyclothone* and others)....Once the postlarvae have entered the metamorphosis stage, they are found almost exclusively at greater depths....After completing the metamorphosis...the little fish, now of an adult appearance, move gradually some way up in the water" (m.w. = metres of wire out).

As an illustration of the youngest stages occurring deepest in the water we may cite the well-known case of the Siphonophore *Velella spirans*, whose larva sinks to deep levels, the parent floating the while on the sea surface (Woltereck, 1904).

Ritter Záhony (1910) appeared to regard temperature as the important factor in the ontogenetic migrations of *Sagitta*. He says of *S. serratodentata* that in the tropics "the young stages are regularly met with. But the older these creatures grow, the less sensitive they become to low temperatures; and they allow themselves on the one hand to be carried by currents into subarctic and subantarctic regions, while on the other hand they sink into the mesoplankton."

It seems most probable that these changes are in reality due to an increasing

or decreasing sensitivity to light so that the optimum intensity becomes lowered or raised as development proceeds. This has been shown to be the case in the laboratory for many animals (see Groom and Loeb, and Mast and Caswell Grave on pp. 242 and 245 of this paper).

Changes in vertical distribution due to spawning habits.

Conklin (1908, *a*), while studying the habits of the Medusa, *Linerges mercurius*, noticed that they appeared very suddenly in large swarms and then disappeared as suddenly. This was not related to ocean currents because it occurred also in relatively enclosed areas.

"When they first appear they come up from deep water, and their disappearance can be seen to be due to their sinking from the surface into greater depths.

"The cause of their sudden disappearance may be observed in the laboratory as well as in nature. The sexual products are shed during the swarming, and after the gonads are emptied the medusae settle to the bottom and soon thereafter begin to disintegrate, as may readily be seen in an aquarium. It can also be observed that the same thing takes place in nature, where many dead or dying medusae may be found over the bottom in shallow water after the swarm has disappeared.

"The movements to or from the surface are not correlated with the intensity of light, as is the case in so many pelagic organisms, for during the swarming these medusae are at the surface at all hours of the day and, so far as I could observe, of the night also. Furthermore they appear at the surface at no other time than the swarming period. It is highly probable that the movement to the surface is correlated with the ripening of the sexual products."

The writer has been unable to find other allusions to such behaviour among plankton animals, but as swarming is of such common occurrence among many species during the spawning period these migrations to the surface may be not unusual

Seymour Sewell (1926, p. 118) says that in the Bay of Bengal it is probable that "at certain definite periods the normal deep-dwelling Salps may approach the surface or even invade the surface water for the purpose of breeding."

Changes in vertical distribution due to hydrographical conditions.

Such changes as may be brought about purely by hydrographical conditions may be regarded as unusual. They will occur only in special regions where there are marked discontinuity layers due to currents of water from different origins overlying one another; these have already been mentioned in the case of the phytoplankton (p. 225), and it may be said that as well as carrying their own flora the different water masses keep their own fauna.

Kramp (1915) gives evidence of *Obelia*, *Sagitta* and other plankton animals, being influenced in their vertical distribution by hydrographical conditions.

Extreme cases, such as the lack of oxygen due to the presence of sulphuretted hydrogen in the deeper waters of the Black Sea (Knipowitch, 1926) have obviously a marked effect on the vertical distribution of plankton animals.

Seymour Sewell (1926) studying Salps in Indian waters noticed a periodicity

in the occurrence of certain species at the surface. Their appearance at the surface was at intervals of about 18 days, coinciding with a rhythmical increase in salinity. In the area in question, the Bay of Bengal, there exist a deep, more saline layer and a superficial less saline, river-polluted layer. Sewell suggests that the periodic changes in surface salinity are due to seiches set up by the periodic changes from N.E. to S.W. monsoons. "Round the margin of each basin or inlet each oscillation will bring the denser water nearer the surface, and by wave action there will be a certain amount of mixing between the layers resulting in a periodic rise and fall in salinity of the surface water itself, and, as I believe, in a rhythmical appearance on the surface of certain species of animals, especially plankton, which normally live at some depth below the surface probably somewhere about the level where the two water strata are in contact."

He also quotes Apstein, who remarked on the disappearance, or at any rate great reduction in numbers, of Salps obtained in the upper layers in the Gulf of Guinea after the influx of fresh water from the Niger or Congo.

Zooplankton may be forcibly brought to the surface layers from deep levels by upwelling of water. Such a phenomenon is well known to occur at times in the Straits of Messina where many deep living plankton organisms can be captured in the upper layers; although even here Lohmann (1910, p. 224) has shown that Appendicularians are able to keep their normal vertical distribution, a fact that should not be lost sight of.

It is necessary here to mention recent work by Helland-Hansen and Nansen (1926). Definite indications are shown that in the sea there are periodic diurnal (or even semidiurnal) vertical oscillations of the water layers. From the few observations so far obtained it seems·that these movements may be as much as 150 m. in 13 hours at a depth of 800 m. and 200 m. in the same time at 1200 m. It is believed that these oscillations may be in some way connected with tidal phenomena, but as yet the observations are too few to conclude. It is as well, however, to keep this phenomenon in view: because, if indeed they are oscillations with a tidal period, we surely have a crucial test to see whether it is temperature or light that limits the vertical distribution of the deeper plankton animals. On an occasion when the oscillation is at its summit at midday the animals if limited by temperature should perhaps show a similar oscillation; if light, however, be the important factor, then as the water layers rise they should be continually altering their level to keep in the same intensity, and collections would show no apparent differences in depth distribution from a day on which the oscillation was at its lowest at noon.

To sum up, the indications are that in the sea each plankton animal may have its own vertical zone in which it finds certain conditions most favourable. This zone varies, not only for different species but for individuals of different ages and stages of development of the same species, and even for the different sexes (Russell, 1927, p. 583). The type of distribution for any one species may vary from place to place, from season to season, and day to day.

It would seem that in fairly homogeneous waters light intensity may be the factor of prime importance governing the distribution of the different species, though other factors such as temperature and salinity within limits may play their part, perhaps in altering the sensitivity of the animal to light. Rate of movement must be an important factor in the various sudden changes in vertical distribution exhibited, and the distribution of food is not to be ignored.

EXPERIMENTAL OBSERVATIONS ON PLANKTON ANIMALS.

Having seen how the various plankton animals behave in nature, as far as is possible from the data we have at present, one wishes to know to what extent these observations have been supplemented by laboratory experiments.

In this review some reference will be made to experiments performed on fresh-water plankton animals. Although very similar in their behaviour, in that they undertake vertical migrations at night, it is necessary to point out that there is an important physiological difference between many small fresh-water and marine animals. In small marine organisms the body fluid has a somewhat similar constitution to the surrounding medium and a quick interchange of some of the dissolved constituents becomes possible; on this account many can stand fairly large changes in concentration or dilution, although they thus become more susceptible to the presence of toxic substances. In fresh-water animals a higher concentration than that of the surrounding medium is normally maintained internally and interchange is thus restricted; they become therefore more susceptible to changes in the surrounding water (Adolph, 1925).

In 1890, Groom and Loeb experimented with the nauplius larva of the acorn barnacle, *Balanus perforatus*, at Naples. They showed that some larvae exhibited positive phototropism and others negative. They also found that light of different wave-lengths had varying effects, the short wave-lengths being the most efficient in making the animals react to light. The sign of phototropism was reversible; if the larvae were kept a long time in darkness they were positive to light, but if the light intensity was increased and grew very bright they became negative once more. From their results they put forward the theory that diurnal vertical migrations could be accounted for entirely by heliotropism. In bright daylight the *Balanus* larvae became negatively heliotropic and therefore moved downwards in the water. During the course of the day they could reach a depth of 30 to 40 m. when the low intensity towards sunset turned them once more positive and they ascended to the surface until daylight next morning. They also explained the seasonal migrations, that Chun had put down to temperature (see p. 232 of this paper), on similar lines. Owing to the increased length of day over the night in summer the period of negative phototropism becomes longer than that of positive, and each day the animals get progressively deeper in the water.

Later, Loeb (1893, *a*), working with *Polygordius* larvae and certain Copepods, probably *Temora longicornis*, included geotropism as well as phototropism in his theory.

Davenport and Cannon (1897) experimented on *Daphnia*, a fresh-water Entomostracan, to find whether:

"1. In phototaxis, the movement of the organisms towards the source of light is primarily a movement in the direction of the light rays, or towards a region of increasing intensity of light.

"2. Whether the *intensity* of the light affects the rate of migration of the phototactic organism."

They found that "Daphnias known to be positively phototactic will move nearly uniformly towards the source of light from a region of greater intensity of light into a region of less intensity along the path of the incoming ray," and that "with a considerable reduction of light there is a slight increase in the time of migration—with ¼ light about 118 % of the time of full light."

Parker (1902) found that both in diffuse daylight and in darkness females of the marine Copepod *Labidocera aestiva* Wheeler kept always at the top of a glass cylinder; he showed that the attraction was not oxygen. He assumed therefore that their geotropism was negative. The males, however, behaved differently; there were two types:

1. Individuals who stayed at the surface with the females.

2. Others scattered uniformly through the water.

When both types were separated from the females they behaved identically, being evenly distributed in the vessel from top to bottom both in light and in darkness.

"...it is the responses to females rather than to other factors that divide the males into two classes. So far as their reactions to light, gravity, etc., are concerned, all males form in reality a single class." "...evident...that light is a very subordinate factor in determining the distribution of the males, if, in truth, it is to be reckoned with at all"...(p. 111).

Having established that the females were negatively geotropic in diffuse light and in darkness, and would hence come to the surface of the sea at night, Parker sought to find the factor that would drive them down in the daytime. He found that mechanical stimulation—by agitation due to wave action - failed to change their characteristic geotropic responses: they were not affected by changes in density. Only if submitted to a temperature above 26° C. was their geotropism sign changed. This temperature is too high to be normally met with by them in the locality in which he was working.

In a large jar kept on the laboratory table female *Labidocera* kept persistently near the top of the water *day and night*, although in another jar floated in the harbour and thus freely exposed to the elements the females made regular migrations, being close to the surface at night and at the bottom in the day. Parker then found that a very intense light turned the female *Labidocera* negatively phototropic, which explained their behaviour in nature. They went into the deep layers in daylight owing to negative phototropism (which overcame their negative geotropism) and came to the surface at night because they were positively phototropic to diffuse light and negatively geotropic.

"In this way the migrations of the females are accomplished, and the only reason why these animals do not carry out similar daily movements in the laboratory is because of the absence of one factor, intense light" (p. 119). He concluded that the males at night followed the females to the surface because of positive chemotropism.

He says that the same does not necessarily apply for other species of Copepods.

Ditlevsen (1907) studied the reactions of various fresh-water and marine plankton organisms with respect to the phenomena of phototaxis and photopathy. Phototaxis (Loeb's phototropism) is the movement of an animal to or away from the source of light, orientated along the path of the beam, irrespective of the intensity it may be moving into: photopathy is movement into regions of stronger or weaker light intensity regardless of the direction of the rays. By placing the animals in aquaria with one half covered with blue glass and the other with red, and also by placing a prism containing 10 per cent. copper ammonium sulphate solution in front of an aquarium and so getting a graded intensity, he concluded from the behaviour of the animals that under these conditions it was not possible to detect purely phototactic movements, but that the animals were obviously photopathic.

Loeb (1908) added to his former theory the influence of CO_2 and of the viscosity of the water, a factor that had been brought prominently into notice by Ostwald (1902). Loeb now explained the daily migrations as being a combination of heliotropism, geotropism, CO_2 content of water and temperature. His original simple heliotropic theory had now become very complicated. A good concise account of this is given by Esterly (1919, p. 8).

Ostwald's theory (1902) of the dependence of vertical migrations on the viscosity of the water should be mentioned here. The viscosity of water, or the frictional resistance to a body moving in the water, varies enormously with the temperature. Ostwald suggested that as the temperature of the water rose in the daytime the viscosity was greatly reduced and the animals sank more rapidly, while at night, on the water cooling, the increased viscosity would aid the vertical movements of the animals and so bring them to the surface. The diurnal changes in temperature in the sea are however extremely slight, one or two degrees at most, and generally much less, and although change of viscosity might certainly affect the powers of movement of the animals through the water it is doubtful whether it can be regarded as a factor of any importance in the vertical migrations of active animals. Ostwald deduced that the upward migration would not predominate until after midnight owing to the slow cooling of the water, whereas actually the migrations start considerably earlier. That viscosity must be of great importance in the vertical distribution of the minute passive floating life, however, there can be no doubt.

Ewald (1912) performed experiments on *Balanus* larvae under the influence of light rays of different composition, and by varying the chemical constituents of the environment. I give his conclusions in his own words (Ewald, 1912, p. 609):

"Concluding from the information gained by my experiments, on the behaviour of free Balanus larvae under normal conditions, it may be supposed that

they react very similarly to other planktonic forms investigated by the present author. After hatching, the larvae swim towards the surface, the strong increase in light causing them to sink down again very soon by inhibition of their loco-motion. Their movements will consist in a continuous alternation of sinking and rising ('periodical locomotion,' Ewald, 1910) caused by successive inhibition and stimulation, without ever necessitating the taking place of negative reaction. This reaction probably constitutes an artificial product of the laboratory. The 'periodical locomotion,' as described in the paper referred to above, causes the animals to maintain themselves in an area of equal illumination throughout the day, taking them gradually up in the evening and down in the morning. In the evening decrease of illumination will slowly shift the position where inhibition due to pro-longed upward locomotion takes place, nearer and nearer the surface, while the reverse is the case for the morning. It is thus unnecessary to assume that the animals constantly change between positive and negative reaction, as was supposed by Loeb. The eminent usefulness of this mechanism is shown by the experiments demonstrating the strong deleterious effect of the light rays of short wave lengths on the nauplii."

Menke (1911) showed a periodic change in the state of the chromatophores in the Isopod *Idothea*, and, in discussing the vertical migrations of the Mysid *Hemimysis lamornae*, suggested that "Eine Analyse verschiedenartiger periodischer Bewegungen im Tierreich, von periodischen Chromatophorenbewegungen einer-seits und von Vertikalwanderungen anderseits, führt zu dem Ergebnis, dass hier wesensgleiche Vorgänge vorliegen. Die Bewegungen sind autonomer Natur."

Esterly (1917, c) demonstrated a physiological rhythm in the Copepods *Acartia tonsa* and *A. clausi*. He found that, if the animals were kept in a tall jar in darkness all day, from 6 p.m to 8 p.m. there was a marked increase in relative numbers in the upper parts of the column of water compared with the lower portions, and not at other times of the day or night. Further, this might be repeated on the second day although the animals had been in darkness all the time. He also brought to light a serious factor to be considered in laboratory experiments (1917, c). *Acartia* collected from the surface showed different reactions to those collected from deeper water; at the same time he found that laboratory conditions might affect their behaviour. This is a serious complication and, as Esterly points out, it con-siderably increases the difficulties of drawing conclusions about natural behaviour from laboratory experiments.

In 1919, Esterly carried out a large number of experiments on various Copepods, and on *Sagitta bipunctata*, in an attempt to correlate laboratory reactions with the natural behaviour of which he had obtained indications by extensive collections in the field. On account of the very real limitations resulting from his discovery of the apparent physiological differences in animals from different depths Esterly is naturally cautious in his views and does not put forward a general theory ex-plaining vertical distribution.

Mast (1921) and Caswell Grave (1920) have worked on the reactions of the tadpole larvae of the Tunicate *Amaroucium*. While Mast's work was mainly con-

nected with the controversy between the "shock reaction" and the De Candolle-Verworn theories on the mechanism of phototropic orientation, both he and Grave showed that when the larvae emerge from the colonies they are at first definitely positive to light for a short time and then for the rest of their free-swimming period they react negatively. Grave found that the larvae at first remained near the surface of the water, but as the time for metamorphosis approached they descended to lower strata (1920, p. 256). This behaviour he interpreted to indicate responses to gravity, negative at first, positive finally.

Grave and Woodbridge (1924) worked on the tadpole larvae of *Botryllus*. As with the larvae of *Amaroucium* the phototropism changed from positive to negative with age. They further showed that during the period of positive phototropism the negative geotropism was the stronger, because if illuminated from below they still remained at the upper surface of the water. The larvae also exhibited a shadow reaction; if a sudden shadow were cast over a sinking larva, when in its positive geotropic stage, it immediately swam actively upwards for a time. They
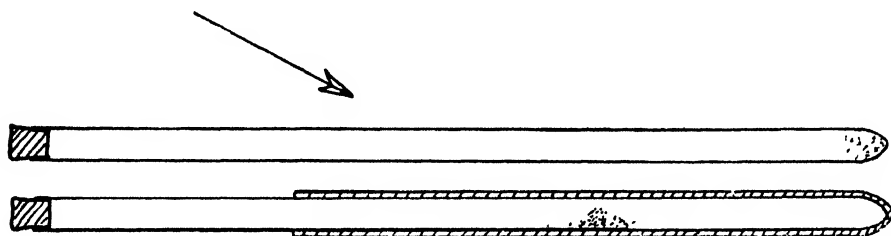
Fig. 6. Showing the distribution of negatively phototropic Copepods in uncovered and covered tubes in strong diffuse light. (After Rose, 1925, Fig. 23, p. 463.) The arrow shows the direction of the light. The lower tube shows the distribution 30 minutes after placing the black cylinder over the tube.

suggested that this was an adaptation to lead the larvae to settle on the under side of *Zostera*, and the indications obtained by experiment were strongly in favour of this.

Eyden (1923), experimenting with the fresh-water Entomostracan *Daphnia*, showed the possible importance of specific gravity in their diurnal behaviour. Working on narcotised animals so that the specific gravity could be interpreted as rate of fall through the water, she showed a periodic change in specific gravity due to feeding.

Fox (1925) found that the Ciliate *Paramecium*, and the larvae of an Echinoderm, *Diadema (Centrechinus) setosum*, apparently had their geotropism affected under different conditions of horizontal illumination. In strong light they swam downwards in the tube and collected at the bottom, in darkness they swam to the surface. Of the different wave-lengths the ultra-violet had the greatest effect.

Rose (1923, 1924, 1925) obtained very significant results by a method that had not hitherto been employed. Up till this time all experiments on phototropism had been conducted in uncovered vessels so that the animals merely went from one end to the other along the path of the rays either towards or away from the

source of light. Rose (1925) employed a tube 1 m. in length lying horizontally on a bench and directed towards the source of light. By means of a black cylinder which would slide over the glass tube he was enabled to produce an intensity gradient within the tube. I figure here (Fig. 6) a result he obtained with negatively phototropic Copepods. When these were placed in an uncovered tube they retired, as was to be expected, to that end farthest from the light. He now enclosed the tube in the black cylinder, about two-thirds the length of the tube, so that the end farthest from the light source was completely sealed. There was now obviously a source of light at the unsealed end and a graded intensity within the covered part of the tube. On slipping the black cylinder on, the Copepods, which had been situated at the end farthest from the source of light, were now under conditions of considerably lowered intensity. They became positively phototropic and moved towards the source of light. "Then rapidly they turned back and ended by placing themselves in a zone of shade corresponding to a given light intensity. If the black cylinder is long enough, one never finds them at the bottom nor at the opening, but in an intermediate region....In fact, it happens as if the animals tend to place themselves in a zone of light intensity particularly clearly defined, to which they would be specially adapted, for the moment at least. The observation shows, besides, that they reach this region and keep themselves there as a result of a series of oscillations, from one part to the other, the amplitudes of which rapidly die out" (Rose, 1925, pp. 463–464).

"This experiment confirms and completes in a manner what we have found on the influence of light intensity on the sign of phototropic reaction. Below a lower limit of intensity the animals are positive; above an upper limit of intensity they are negative: between these two intensities they are indifferent. And it seems that their reactions towards the light tend to bring them to this zone of indifference."

In this long and interesting paper (1925) Rose reviews previous work very fully and expresses his own ideas as to the causes of diurnal migration. He gives a bibliography of 109 titles. He places the factors in the following order of importance:

1. Light, which under average conditions has clearly a predominating influence.

2. Temperature, which becomes very important and can even overwhelm the effect of light when it passes 20°.

3. Other factors of the medium (concentration, aeration, etc.).

He concludes that each species and even each individual and stage of development has its own optimum light intensity at which it lives, and that by a mechanism consisting of "*phototropisme*, les *sensibilités différentielles* et des réactions d'adaptation à des intensités optimum d'excitation du groupe des *pathies*" (p. 537) it keeps at the required level. Rose does not favour the use of the word geotropism and thinks that the question of a physiological rhythm must await further evidence.

Russell (1926, *c*) compared the actual distribution shown by *Calanus finmarchicus* in the sea with that which would be theoretically expected to occur if light intensity were the main controlling factor. Assuming that the Copepods had an optimum intensity at which they lived and that they could exist within a certain

range of intensities so that they had an upper and a lower limit, and assuming that these maximum and minimum limits were equidistant in light intensity units from the optimum, it would appear that the vertical distribution of an animal capable of enduring a fairly long range of intensities should, on account of the manner in which light is absorbed in sea water, be like that shown as *B* in Fig. 7. Now in Fig. 8 is given the actual distribution shown by *Calanus* female adults obtained in collections made on April 13th, 1926, near Plymouth. The similarity between the theoretical distribution and the observed distribution is striking, and, what is more, it is of common occurrence, although the optimum may not necessarily
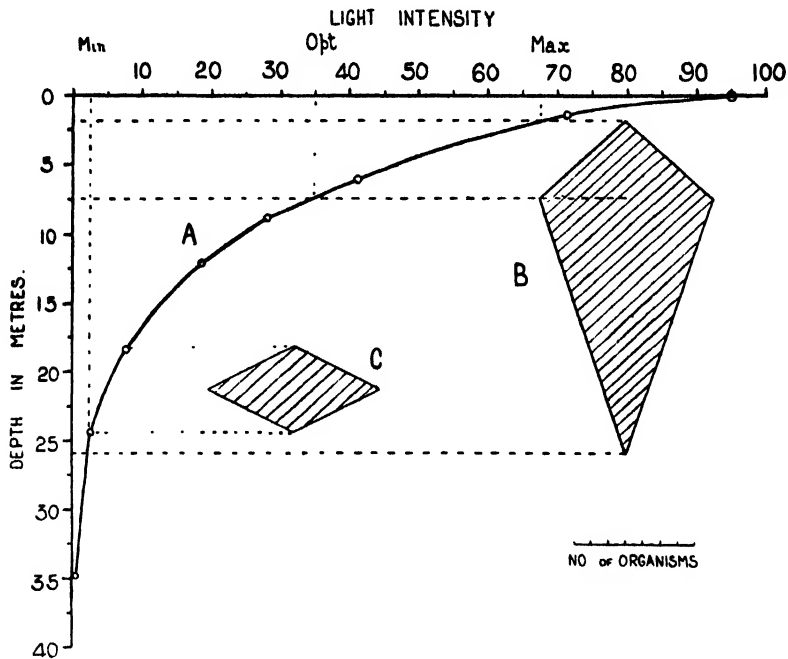
Fig. 7. *A* = actual curve of light penetration into sea water obtained by Poole and Atkins (1926). *B* = Theoretical vertical distribution of a species that can withstand a long range of intensities; this should be compared with Fig. 8. *C* = Theoretical vertical distribution of a species that lives within a narrow range of intensities. (After Russell, 1926, *c*. Fig. 3, p. 421. By kind permission of the Marine Biological Association.)

be exactly midway between the maximum and minimum. Russell further showed other changes in vertical distribution that could be correlated with seasonal changes in light intensity and daily weather conditions.

I give below in summary form some of the various other reactions noted by the workers cited above.

Effects of temperature.

Loeb (1893, *a*). Warming makes *Polygordius* larvae and Copepods, probably *Temora longicornis*, negatively heliotropic. Cooling makes the same species positively heliotropic.

Parker (1902). Raising the temperature to 30 and 35° C. converts *Labidocera aestiva* females from negatively geotropic to positively geotropic.

Ewald (1912). "Increase of temperature made positive animals negative and negative animals more negative, and that decrease of temperature made negative animals positive and positive animals more positive....The amount of the change in temperature necessary for the reversing of the reaction to light depends on the age and state of the larvae." This refers to *Balanus* larvae.

Esterly (1919, p. 31). *Calanus finmarchicus* "is negative to light of all intensities
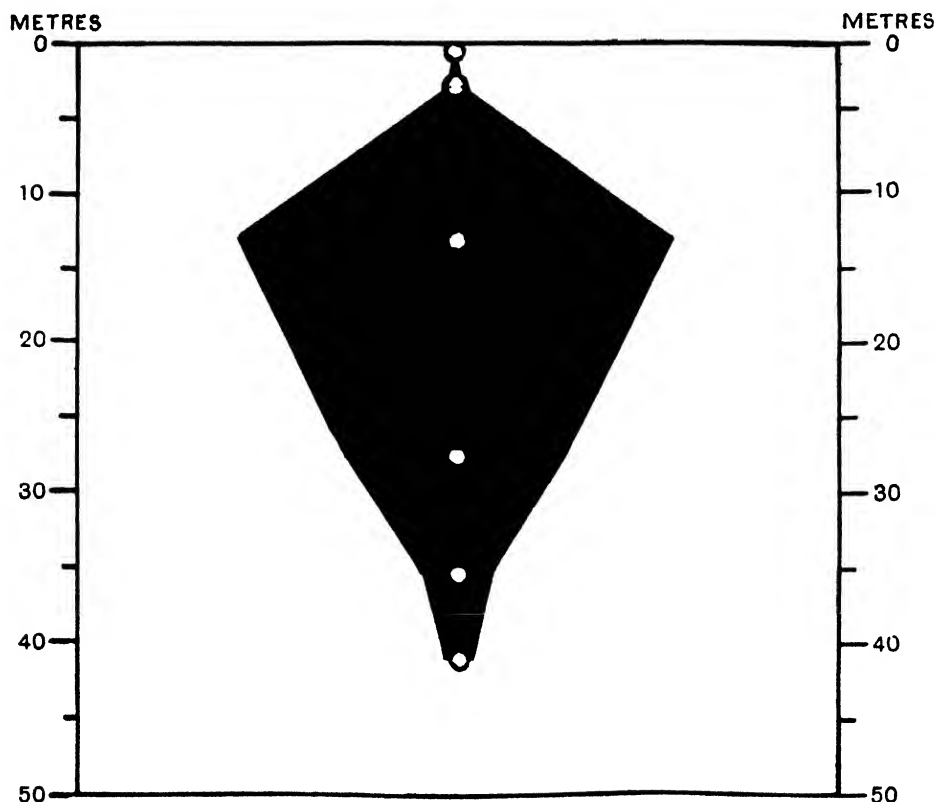
Fig. 8. Percentage vertical distribution of female *Calanus finmarchicus* adults in the English Channel off Plymouth on April 13th, 1926. The white spots indicate the six depths at which collections were made. The percentage scale is 1 cm. = 10 %. This figure should be compared with *B* of Fig. 7.

except when the water is cooled. The phototropism becomes noticeably positive at about 13° C., and is pronouncedly so at temperatures below 10° C."

(p. 50.) *Metridia lucens* "is negative to each of these intensities at room temperatures and in water of usual salinity. Positive responses predominate, however, if the water is cooled to 10° C."

Rose (1925, p. 465). *Acartia clausi*—a strongly positively phototropic form: positive phototropism disappears only at 28° C.

Centropages hamatus—positive until temperature reaches 25° C., above this it becomes more and more indifferent and even negative.

Temora longicornis, Parapontella brevicornis, and *Isias clavipes*, already become rapidly negative at 22° C.

It is interesting to note that of these forms *Acartia* is a surface-living form in nature, *Centropages* lives deeper and *Temora* is deep living (Russell, 1926, c).

Effects of different chemicals.

Loeb (1893, c). *Polygordius* larvae and Copepods made positively phototropic by increase of concentration, and negative by decreasing the concentration by adding fresh water to sea water.

Loeb (1906). *Balanus* larvae become positively phototropic on addition of CO_2 to water.

Ewald (1912). Isotonic NaCl solution added turns negative animals positive, and positive animals more positive. Isotonic KCl solution produces the same result, but is less effective. The addition of isotonic calcium chloride solution makes the larvae lose their power of reaction to light, causing them to swim at random without negative or positive reaction. "Magnesium chloride or sulphate solution acts as an antagonist to sodium." "For a normal production of light reactions it is necessary to have the correct proportion of sodium on the one side and magnesium on the other." "Lack of oxygen, brought about by evacuating the sea water, had a very strong positivating effect." All these observations refer to the larvae of *Balanus*.

Moore (1912) found that negative phototropism produced in the fresh-water Crustacean *Daphnia* by the action of ultra-violet rays was reversed by the addition of small quantities of CO_2 or HCl.

Esterly (1919). "The reactions of *Calanus* to light do not change when the salinity is increased."

Rose (1925, p. 465) studied the effects of many chemicals on different plankton animals. Briefly his results on Copepods were: Sufficient dilution of the sea water reversed phototropism from positive to negative. Strongly positive forms such as *Acartia clausi* were most easily reversed, but *Centropages hamatus*, a less strongly positive form, was more resistant.

Increasing the concentration strengthens positive phototropism, but negative Copepods lose their sensitivity and die very quickly in water evaporated to four-fifths of its original volume.

Acids, such as acetic, hydrochloric, sulphuric and carbonic, did not show very obvious action on the phototropism. The apparent indifference of the marine Copepods to CO_2 in comparison with fresh-water animals such as *Daphnia* was striking and contrary to the generalised effect that Loeb suggested and employed in his theory of vertical migration (p. 244). "Alkalis appear a little more efficacious than acids, without having however an intense action." They tend to reverse positive forms. For the effects of changes in hydrogen-ion concentration Rose says (p. 474): "The feeble variations of the pH of the water in which they live has on

their phototropism only a very feeble action, practically negligible. The natural fluctuations in pH, always of small importance in ordinary conditions, ought to have no effect on their phototropic reactions. But, with strong artificial variations, one can see a pronounced influence. The maximum sensitivity to light appears very clearly in the neighbourhood of neutrality."

Of the action of salts he says: "In a general manner the salts tried were without clear action on the phototropic reactions of our copepods. We have experimented with a large number, sulphates, alkaline nitrates, chlorides, etc., without clear and constant results. Only perhaps are the effects of potassium and sodium chlorides worthy of mention. In effect, they appear to have an antagonistic action, the former very toxic, rendering insensitive the positive forms, the second making them sensitive....In water deprived of its calcium by an oxalate the positive forms are at first obviously rendered insensitive. Then the crustacea present intensely convulsive movements, whirl around and die very quickly."

Rose further experimented with oxidising reagents, reducing reagents, sugars, alcohol, picric acid, anaesthetics and alkaloids.

Fox (1925) showed that the "addition of acid to the sea water causes upward, of alkali downward, movement" in echinoid larvae: bubbling through of CO_2 caused subsequent upward migration.

Deleterious effects of ultra-violet radiation.

Ewald (1912, p. 597) placed two blackened watch-glasses containing *Balanus* larvae in diffused daylight or sunlight. He covered one with a strong clean glass plate and the other he left uncovered. After a few minutes in sunlight, or one or two hours in strong diffuse light, the nauplii in the uncovered dish were killed. Those in the covered dish lived for hours.

Moore (1912, p. 574) showed that glass cut off the ultra-violet rays shorter than 3341 Å.U., and that the rays thus cut off were specific for causing negative phototropism in *Daphnia pulex*. Negative phototropism so produced was reversed when small quantities of CO_2 or of HCl were added to the water containing the animals.

Huntsman (1924) showed the harmful effect of sunlight on lobster larvae, deep-water planktonic forms, such as *Calanus finmarchicus* and *Meganyctiphanes norvegica*, and also on shallow-water plankton forms such as *Acartia clausi, Pseudocalanus elongatus* and *Temora longicornis*. He says: "These experiments demonstrate the fatal effect of sunlight on certain marine animals. The light may be more effective at certain stages in the life cycle than at others, as would seem to be the case for the lobster." It is interesting to note also that he found that Meganyctiphanes "that had been retained in the laboratory without protection from light for eleven days, proved quite resistant to direct sunlight....The unfavourable effect of the light thus depends upon the condition of the individuals as well as upon the strength of the light."

DISCUSSION.

The writer must confess that he has, as yet, undertaken no experimental study of the behaviour of plankton animals under laboratory conditions. With the usual guardedness of the field observer he therefore feels somewhat diffident about putting into concise statements his theoretical ideas. Nevertheless, it is a fitting conclusion to an article of this type to give a discussion, and the following is written on the understanding that it is fully realised that future work may considerably alter the ideas. It is hoped, at least, that it may serve to point the way to lines of future research.

We see that the early workers, such as Weismann (1876), Fuchs (1882) and Chun (1888), based their theories on only a slight knowledge of the behaviour of animals in nature and on the observed changes in the natural medium. Later, Loeb, as a laboratory experimenter purely, brought forward his simple heliotropistic theory, which he later considerably modified by the inclusion of other types of tropisms.

At the present time we have a considerable body of experimental evidence to draw from and our knowledge of the vertical distribution and behaviour of animals in the sea is much increased, though many more accurate observations are yet to be desired. As so often happens it is probable that the theory put forward by each worker had something of the truth in it, and by sifting these points from each theory and building up a new one we shall most likely reach the nearest possible solution at the moment.

That light is evidently a factor of great importance seems to be in common agreement; but we have seen that there has been considerable difference in opinion as to how it acts. Some have postulated that it brings into play a movement either towards or away from the source of light, *i.e.* positive and negative phototropisms. Others suggest that it sets up reactions to gravity causing the animal to move against the force of the earth's attraction and so rise, or to allow gravity itself to act and so sink, either passively under its own weight or by active downward swimming, *i.e.* negative and positive geotropism. Another thinks that the different intensities of light may stimulate or inhibit locomotion in the animal.

It seems most probable that light can be regarded as the most important controlling factor—the ultimate decider. The movements set up, whether phototropic, geotropic or otherwise, must then be primarily dependent on photochemical reactions taking place within the organism.. Thus it is very generally found that a rise in temperature will turn positively phototropic animals negative, or make negative animals more negative, which can best be interpreted by imagining a speeding-up of photochemical reaction and consequent apparent increased sensitivity to light conditions; conversely, a drop in temperature for the same reason changes negative phototropism to positive. In nature it will be found that such reactions are well adapted to existing conditions. A high temperature, setting up a negative phototropism, will cause the animal to retire into deeper and cooler layers. In so doing the force of gravity plays its part. Now a true phototropic reaction requires that

the animal shall travel either towards or away from the source of light along the path of the beam, positive animals heading towards the light and negative ones pointing away from the source; such can be easily seen if some plankton animals are kept in a flat dish near the window. It seems unlikely that in nature such animals as Copepods normally, when negative to light, swim head downwards; it is probably more usual for them to sink slowly tail first with outspread antennae (Esterly, 1919, p. 31; Parker, 1902, p. 106), although, according to Esterly, *Calanus* "under certain conditions will *swim* down," and (1911, p. 149) he was inclined to conclude that they swim down in nature, while Parker says that the males of *Labidocera* often swim downwards. Such sinking is not true negative phototropism according to Loeb's definition; if such were the case then in nature true negative phototropism would not exist in the normal state of affairs in open waters. It would rather be inhibition of locomotion.

Now it has been shown for certain forms that, while high intensities will set up negative phototropism and low intensities positive phototropism, there is between the high and low intensities a neutral ground and a certain intensity at which the animal is apparently indifferent, showing no phototropism. Evidence has been brought forward both in the laboratory (Rose, see p. 247 of this paper) and in nature (Russell, see p. 247 of this paper) that certain animals appear to have an optimum intensity at which they live, and at the same time upper and lower limits of intensity within the confines of which the population remains, the majority being in the region of optimal intensity. This can be simply explained by the fact that although many of the population are in the region of optimum conditions, random movements, such as excursions after food, will tend to draw others away from this area. As the animals get further from the optimum towards the upper limiting intensity they will become increasingly stimulated owing to increased speed of photochemical reaction under the increasing illumination. There will thus be a driving force tending to keep them down to the optimum region, which increases in strength the nearer they approach the upper limit. Similarly, as the animals extend out towards the lower limit of their intensity range the force driving them upwards will get stronger.

The writer is not going to generalise on the actual method by which the animals keep round the optimum. It may be phototropism, geotropism or acceleration and inhibition of motion. It may be a combination of all. From the evidence of laboratory experiments it may differ for different animals; in some, phototropism has been shown to overpower geotropism, *e.g. Labidocera* females (Parker, 1902); in others the effect of geotropism appears to be more powerful than that of phototropism, *e.g. Botryllus* larvae (Grave and Woodbridge, 1924). It seems, however, that it would be rather hard to draw the line between the two in nature since they act in exactly opposite directions, and a downward movement may as well be interpreted as negative phototropism as positive geotropism. The important point is that the mechanism, whatever it may be, brings the animals into their region of optimum illumination; and by illumination is meant composition as well as intensity of light, owing to the selective absorption of the water.

Although such other factors as temperature may be regarded as limiting factors in nature, it seems probable that, within certain limits, the ultimate factor is the light, but that owing to change in temperature the photochemical reactions may change in speed and the animals become more, or less, sensitive. This, of course, excludes such high or low temperatures as may upset the general metabolism of the animal and lead to its death.

Before concluding it would seem that, on account of the apparent very close connection between the behaviour of certain plankton animals and the light *intensity* conditions, one or two points are worthy of mention.

1. Before studying the reactions of animals in the laboratory it is necessary to know precisely the normal conditions under which they live in nature. Since light is evidently one of the most important factors governing their behaviour, the normal light intensity under which they exist should be determined and the animals should be kept under these conditions until experiments are started. To cite an instance, Esterly (1919, p. 22) says: "All the animals that were used in experiments were obtained at sixty to one hundred metres"; and further (*ibid.* p. 23), it seems that collecting generally took place in broad daylight. To illustrate my point I will suppose that one wishes to test the reactions of the Medusa *Cosmetira pilosella*. In the daytime this Medusa always lives, in the region of Plymouth, at a depth below 20 m., the level of its maximum abundance lying at about 25 m. (Russell, 1927, p. 572). The intensity of light in that locality at midday on October 1st, 1925, at 24·4 m. was 470 metre-candles (Poole and Atkins, 1926, p. 192) and, on September 3rd, 780 metre-candles at 27·2 m. We do not know the actual intensity at this depth in the summer, but we can suppose it will be not more than 2000 metre-candles. It will then be probable that in nature *Cosmetira* throughout its whole life never experiences a higher intensity than this, only coming towards the surface as the light diminishes in the evening (Russell, 1926, *c*, p. 432). If these animals be captured in the daytime they will possibly be brought up to a light intensity of, may be, 80,000 metre-candles, or more, on the deck of the ship. It is difficult to imagine the effect that such an intensity may produce on an animal whose whole physiological and morphological structure is presumably made up to live only at the low intensities of 2000 metre-candles or less. Collecting should surely take place at night, and during the whole course of experiments to examine the *normal* reactions of the animal it should never be allowed to experience an intensity much greater than 2000 metre-candles if one wishes to draw conclusions on the behaviour of an average individual of that species.

2. If it is indeed a fact that for each species there is an optimum intensity, the strength of illumination under which experiments are carried out should if possible be determined, so that correlations may be made with the differing intensities under which they exist in nature.

3. Esterly (1919, pp. 39–41) discusses the bearing of his experimental evidence on the diurnal migrations. He is unable to account for the fact that in nature *Calanus* is on the surface in darkness, whereas in the laboratory "there is practically no upward movement in darkness or in diffuse light if the observations are made

during the hours of daylight." He says: "An explanation of the diurnal migration of *Calanus* based on geotropic and phototropic reactions is beset by too many difficulties to be readily acceptable in view of the results of field work. The matter of the physiological rhythm affords a simpler way of accounting for the habit, but it ought to be supported by more evidence than that brought forward here."

On the theory outlined on p. 237 these difficulties are removed; the *Calanus* only reach the surface by following their optimum intensity upwards as daylight wanes; in darkness all light stimulus is removed and the Copepods are free to go where they like. It is therefore natural that in the laboratory we shall find that darkness has no power to make the animals move towards the surface.

4. The question of a physiological rhythm is of interest. It seems, however, almost to be expected that such should occur, but it is, of course, brought on primarily by the periodic changes of the environment. The physiological rhythm is not responsible for the diurnal movements; it is the environmental changes that bring about the periodic movements and thereby set up in the animal a physiological rhythm. It is therefore natural to expect such "habits" to show themselves for a period in the laboratory after the stimuli of the external environment have been removed. One would not imagine then that the physiological rhythm is of much importance in nature except in that it makes the animal "ready" for the environmental changes when they occur.

Walther (1893, p. 173) says that "A. Walter observed that the current in the Hinlopen Strait at Spitzbergen ran in a southerly direction. In this current lived many Medusae (*Codonium, Hippokrene, Catablema*) which Walter caught from early morning to eight o'clock in the evening only at 30–80 metres. From nine in the evening until six in the morning these animals were swimming at the sea surface. This fact does not seem so wonderful when we consider that most plankton animals of warmer regions sink into the depths of the sea in the daytime and towards night come to the surface. And because the current of the Hinlopen Strait is the last arm of the Gulf Stream, the periodic migration of the plankton seems easy to understand.

"However at Spitzbergen the day is uninterrupted in the summer; the midnight sun shines from early summer to autumn, and the periodic wandering of the plankton is only to be explained through the fact that those Gulf Stream Medusae, which were brought from southern parts of the sea to the land of the midnight sun, still adhere with great tenacity in their new home to the apparently completely purposeless habit, which they knew to be profitable only in the tropics."

Römer (1904) confirmed these observations on Ctenophores.

These facts have been regarded by Loeb (1893, *b*, p. 67) and Menke (1911, p. 81) as possible evidence of a physiological rhythm or "habit" in nature.

The argument is not convincing. At Spitzbergen, about lat. 80° N., with a mean declination of 20° N. for June the sun's altitude at midday would be about 30°, while at midnight it would be only 10° above the horizon. This means that while the percentage loss of illumination by reflection from the sea's surface is only about 40 per cent. at noon, at midnight it is nearly 90 per cent. Add to this the smaller number of rays incident on a unit of horizontal area owing to their greater

obliquity at midnight compared with that at noon and the diurnal change in light intensity (Mohn, 1905, p. 594, gives the sum of the sun's radiation in lat. 83° 6' for June as 1·223 gram. cal. per sq. cm. per min. at noon and as only 0·590 at midnight). There is surely sufficient evidence of a diurnal variation in light intensity.

This would appear to be confirmed by a statement made by Römer (1904, p. 72) to the effect that in the warmer, ice-free waters of the west coast the difference between the scarceness of plankton in the surface layers in the daytime, and its abundance in the evening and at night, is much more marked than below the thick pack-ice of the eastern region. It is most probable that this is due to the strong cutting off of the light rays by the ice.

5. The question has been raised by Franz (1913, p. 264, quoted from Esterly, 1919, p. 72) whether the light conditions beneath the surface are those of diffuse light, and that for this reason phototropic reactions may not be brought about normally in nature. It should be at once pointed out that this is not the case; light is most certainly directional; this has been demonstrated by Helland-Hansen to be the case at a depth of 500 m. in tropical waters (Murray and Hjort, 1912, p. 252).

6. There was a fact that puzzled the writer for long when first he took up the study of vertical distribution; this was, how is it that certain animals may be positively phototropic on a laboratory bench when we know that in the sea they avoid the surface in the daytime? This question became at once answered when it was realised that the light intensity in a room a few feet from a north window was usually only about 1 per cent. of the intensity out of doors. *Calanus*, for instance, may have as its optimum intensity in nature as much perhaps as 10,000 metre-candles (this is a rough guess merely, it is hoped in time that sufficient data will be forthcoming to indicate the various intensities at which the different animals live). It is obvious then that in the laboratory the intensity may be well below its optimum, which would account for its positive phototropism.

In conclusion I wish to state that I do not claim originality for many of the statements made above. It is impossible to be continually referring to different authors' opinions, and many of the ideas have been already suggested from time to time by the various workers quoted. It is rather by examining each one's opinion, making modifications and piecing the whole together, aided by indications obtained from field observations, that I have set out the above suggestions; and it is therefore to the many previous workers in this field of research that my thanks are due.

BIBLIOGRAPHY.

This bibliography, in conjunction with those of Allen (1926), Steuer (1910) and Rose (1925), should cover most of the literature dealing with the vertical distribution of plankton organisms and the problems that arise therefrom.

ADOLPH, E. F. (1925). "Some physiological distinctions between freshwater and marine organisms." *Biol. Bull.* **48**, 327–335.

ALLEN, E. J. (1926). "A selected bibliography of marine bionomics and fishery investigation." *Journ. Conseil Intern. pour l'Explor. de la Mer*, **1**, Nos. 1 and 2.

ALLEN, E. J. and NELSON, E. W. (1910). "On the artificial culture of marine plankton organisms." *Journ. Mar. Biol. Assoc.* N.S. **8**, No. 5, 421–474.

APSTEIN, C. (1906). "Plankton in Nord- und Ostsee auf den deutschen Terminfahrten." I. Teil. (Volumina 1903). *Wiss. Meeresunter.* Kiel, N.F. **9**, 1–26.

—— (1910). "Hat ein Organismus in der Tiefe gelebt, in der er gefischt ist?" *Intern. Rev. Hydrob. Hydrogr.* **3**, 17.

ATKINS, W. R. G. (1925). "On the thermal stratification of sea water and its importance for the algal plankton." *Journ. Mar. Biol. Assoc.* N.S. **13**, No. 3, 693–699.

—— (1926, *a*). "The phosphate content of sea water in relation to the growth of the algal plankton." *Journ. Mar. Biol. Assoc.* N.S. **14**, No. 2, 447–467.

—— (1926, *b*). "A quantitative consideration of some factors concerned in plant growth in water. Part I. Some physical factors. Part II. Some chemical factors." *Journ. Conseil Intern. pour l'Explor. de la Mer*, **1**, No. 2, 99–126, and No. 3, 197–226.

ATKINS, W. R. G. and POOLE, H. H. (1926). "The distribution of red algae in relation to illumination." *Nature*, **118**, No. 2961, 155–156.

BAUER, V. (1909). "Vertikalwanderung des Planktons und Phototaxis." *Biol. Centralb.* **29**.

BERTEL, R. (1912). "Sur la distribution quantitative des bactéries planctoniques des côtes de Monaco." *Bull. de l'Inst. Océanograph. Monaco*, No. 224.

BIGELOW, H. B. (1909). "The Medusae. Reports on the scientific results of the Expedition...by the U.S. Fish Commission Steamer 'Albatross'...1904...1905. No. XVI." *Mem. Mus. Compar. Zool. Harvard Coll.* 1–243.

—— (1911). "Fishes and Medusae of the intermediate depths. A note on the work of the 'Michael Sars.'" *Nature*, **86**, No. 2171, 483.

—— (1926). "Plankton of the offshore waters of the Gulf of Maine." *Bull. Bur. Fish. Washington*, **40**, 1924, Pt. II, Document No. 968, 1–509.

BRAUER, A. (1906). "Die Tiefseefische." *Wissen. Ergebnisse der deutschen Tiefsee-Expedition auf dem Dampfer 'Valdivia,' 1898–1899*, Bd. **15**, Lief. 1, Jena.

BROWNE, E. T. (1910). *National Antarctic Expedition, 1901–1904. Natural History*, **5**, Zoology and Botany, Coelentera, V. Medusae, pp. 1–62.

BURFIELD, S. T. and HARVEY, E. J. W. (1926). "The Chaetognatha of the 'Sealark' Expedition." *Trans. Linn. Soc. London*, 2nd Ser. *Zool.* **19**, Pt. 1, pp. 93–119.

CHUN, C. (1888). "Die pelagische Thierwelt in grosseren Meerestiefen und ihre Beziehungen zu der Oberflächenfauna." *Bibliotheca Zoologica.* Cassel, pp. 1–66.

—— (1890). *Die pelagische Thierwelt in grössen Meerestiefen.*

—— (1897). *Die Beziehungen zwischen dem arktischen und antarktischen Plankton.* Stuttgart, pp. 1–64.

CONKLIN, E. G. (1908, *a*). "The habits and early development of Linerges mercurius." *Papers from the Tortugas Lab. of the Carnegie Inst. of Washington*, **2**. Public. No. 103, VI, 155–170.

—— (1908, *b*). "Two peculiar actinian larvae from Tortugas, Florida." *Papers from the Tortugas Lab. of the Carnegie Inst. of Washington*, **2** Public. No. 103, VI.

DAHL, F. (1894). "Über die horizontale und vertikale Verbreitung der Copepoden im Ozean." *Verh. deutsch. Zool. Ges.*

DAMAS, D. and KOEFOED, E. (1907). "Le plankton de la mer du Grönland." *Croisière Océanographique accomplie à bord de la Belgica dans la Mer du Grönland.* Brussels, pp. 347–427.

DAVENPORT, C. B. and CANNON, W. B. (1897). "On the determination of the direction and rate of movement of organisms by light." *Journ. Physiol.* **21**, 22–32.

DITLEVSEN, H. (1907). "Versuche über das Verhältnis einiger Planktontiere gegenüber Licht." *Skand. Arch. Physiol.* **19**, 241–261.

ESTERLY, C. O. (1911). "Diurnal migrations of *Calanus finmarchicus* in the San Diego region during 1909." *Intern. Rev. Hydrobiol. Hydrogr.* Bd. **4**, 140–151.

—— (1912). "The occurrence and vertical distribution of the Copepoda of the San Diego region." *Univ. Calif. Public. Zool. Berkeley*, **9**, No. 6, 253–340.

—— (1914). "A study of the occurrence and manner of distribution of the Ctenophora of the San Diego region." *Univ. Calif. Public. Zool. Berkeley*, **13**, No. 2, 21–38.

—— (1914). "The vertical distribution and movements of the Schizopoda of the San Diego region." *Univ. Calif. Public. Zool. Berkeley*, **13**, No. 5, 123–145.

—— (1917, *a*). "Specificity in behaviour and the relation between habits in nature and reactions in the laboratory." *Univ. Calif. Public. Zool. Berkeley*, **16**, No. 20, 381–392.

—— (1917, *b*). "Field research and laboratory experiment: their places in ascertaining and explaining habits in nature." *Bull. Scripps Inst. Biol. Research, Univ. Calif.* No. 4, 1–15.

—— (1917, *c*). "The occurrence of a rhythm in the geotropism of two species of plankton Copepods when certain recurring external conditions are absent." *Univ. Calif. Public. Zool. Berkeley*, **16**, No. 21, 393–400.

—— (1919). "Reactions of various plankton animals with reference to their diurnal migrations." *Univ. Calif. Public. Zool. Berkeley*, **19**, No. 1, 1–83.

EWALD, W. F. (1912). "On artificial modification of light reactions and the influence of electrolytes on phototaxis." *Journ. Exper. Zool.* **13**, No. 4, 591–612.

EYDEN, D. (1923). "Specific gravity as a factor in the vertical distribution of plankton." *Proc. Camb. Philos. Soc. Biol. Sci.* **1**, No. 1, 49–55.

FARRAN, G. P. (1910). "Résumé des observations sur le plankton. Copepoda." *Cons. Perm. pour l'Explor. de la Mer. Extrait du Bulletin Trimestriel*, 1902–1908.

—— (1926). "Biscayan plankton collected during a cruise of H.M.S. 'Research,' 1900. Pt. XIV. The Copepoda." *Journ. Linn. Soc. Zool.* **36**, No. 243, 219–310.

FISCHER, B. (1894). "Die Bacterien des Meeres." *Ergeb. der Plankton-Expedition*, Bd. **4**.

FOWLER, G. H. (1896–8). "Contributions to our knowledge of the plankton of the Faeroe Channel." *Proc. Zool. Soc. London*, 1896, p. 991; 1987, pp. 523 and 803; 1898, p. 540.

—— (1905). "Biscayan plankton collected during a cruise of H.M.S. 'Research,' 1900. Pt. III. The Chaetognatha." *Trans. Linn. Soc. London*, 2nd Ser. *Zool.* **10**, Pt. 3, 55–87.

—— (1906). "The Chaetognatha of the Siboga-Expedition." *Siboga-Expeditie*, **21**, 1–86.

—— (1907). *National Antarctic Expedition*, 1901–1904. *Natural History*, **3**, Zoology and Botany, Chaetognatha, pp. 1–6.

FOX, H. M. (1925). "The effect of light on the vertical movement of aquatic organisms." *Proc. Camb. Philos. Soc. Biol. Sci.* **1**, No. 4, 219–224.

FRANZ, V. (1910–11). "Phototaxis und Wanderung. Nach Versuchen mit Jungfischen und Fisch-larven." *Intern. Rev. Hydrobiol. Hydrogr.* Bd. **3**, H. 3/4, pp. 306–334.

—— (1913). Die phototaktischen Erscheinungen im Tierreiche und ihre Rolle im Freileben der Tiere. *Zool. Jahrb. Abth. f. allg. Zool. u. Physiol. der Tiere*, **33**, 259–286.

FUCHS, T. (1882). "Beiträge zur Lehre über den Einfluss des Lichtes auf die bathymetrische Verbreitung der Meeresorganismen." *Verhand. K. K. Geol. Reichsanstalt Wien.*

GAARDER, T. and GRAN, H. H. (1927). Investigations of the production of plankton in the Oslo Fjord. *Cons. Perm. Internat. pour l'Explor. de la Mer. Rapports et Procès-Verbaux*, **42**, 1–48.

GAIL, F. W. (1918). "Some experiments with Fucus to determine the factors controlling its vertical distribution." *Publ. Puget Sound Biol. Stat.* **2**, 139–151.

GAMBLE, F. W. (1909). "The Radiolaria." In *A Treatise on Zoology*, edited by Sir Ray Lankester, Pt. I.

GEHRKE, J. (1909). "Ueber Farbe und Durchsichtigkeit des Ostseewassers." *Cons. Perm. Intern. pour l'Explor. de la Mer. Publications de Circonstance*, No. 45.

GIESBRECHT, W. (1892). "Pelagische Copepoden." *Fauna und Flora des Golfes von Neapel*, Bd. **19**.

GOUGH, L. H. (1905). "Report on the Plankton of the English Channel in 1903." *Mar. Biol. Assoc. Intern. Fish. Invest.* 1902–3. *Southern Area.*

GRAN, H. H. (1910). "Das Plankton des Norwegischen Nordmeeres." *Rep. Norweg. Fish. Mar. Invest.* **2**.

—— (1912). "The plankton production of the North European waters in the spring of 1912." *Cons. Intern. pour l'Explor. de la Mer. Bulletin Planktonique.*

—— (1919). "Quantitative investigations as to the phytoplankton and pelagic Protozoa in the Gulf of St Lawrence and outside the same." *Canadian Fisheries Expedition*, 1914–1915, 489–495.

GRAVE, CASWELL. (1920). "Amaroucium pellucidum (Leidy) form constellatum (Verrill). I. The activities and reactions of the tadpole larvae." *Journ. Exper. Zool.* **30**, No. 2, 239–257.

—— (1924). "Continuation of the study of the organization and behaviour of tunicate larvae." *Carnegie Inst. Washington, Ann. Rep. Tortugas Lab.* 189.

GRAVE, CASWELL and WOODBRIDGE, H. (1924). "Botryllus Schlosseri (Pallas); the behaviour and morphology of the free-swimming larva." *Journ. Morph.* **39**, 207–247.

GREIN, K. (1914). "Untersuchungen über die Absorption des Lichts im Seewasser." Zweiter Teil. *Ann. de l'Inst. Océan.* Tome **6**, Fasc. 6.

GROOM, T. T. and LOEB, J. (1890). "Der Heliotropismus der Nauplien von *Balanus perforatus* und die periodischen Tiefenwanderungen pelagischer Tiere." *Biol. Centralb.* **10**, 160–177.

HAECKER, V. (1908). "Tiefsee-Radiolarien." *Wiss. Ergeb. Deutsch. Tiefsee-Expedition auf dem Dampfer 'Valdivia,'* 1898–1899, Bd. **14**, Jena.

HAECKEL, E. (1887). "Report on the Radiolaria collected by H.M.S. 'Challenger' during the years 1873–1876." *Zoology*, **18**.

HARVEY, H. W. (1923). "Hydrographic features of the water in the neighbourhood of Plymouth during the years 1921 and 1922." *Journ. Mar. Biol. Assoc.* N.S. **13**, No. 1, 225–235.

—— (1926). "Nitrate in the sea." *Journ. Mar. Biol. Assoc.* N.S. **14**, No. 1, 71–88.

HEDLEY, C. (1925). "An opacity metre." *Trans. Roy. Geograph. Soc. of Australia (Queensland). Rep. Barrier Reef Committee*, **1**, No. 10, 67.

HELLAND-HANSEN, B. and NANSEN, F. (1926). "The Eastern North Atlantic." *Geofysiske Publikasjoner, Oslo*, **4**, No. 2, 1–76.

HICKLING, C. F. (1925). "Notes on Euphausiids." *Journ. Mar. Biol. Assoc.* N.S. **13**, No. 3, 735–745.

HUNTSMAN, A. G. (1919). "Some quantitative and qualitative plankton studies of the Eastern Canadian plankton." *Canadian Fisheries Expedition*, 1914–1915, 405–485.

—— (1924). "Limiting factors for marine animals. 1. The lethal effect of sunlight." *Contrib. to Canadian Biol.* N.S. **2**, No. 4.

—— (1924). "Limiting factors for marine animals. 2. Resistance of larval lobsters to extremes of temperature." *Contrib. to Canadian Biol.* N.S. **2**, No. 5.

HUNTSMAN, A. G. and SPARKS, M. I. (1924). "Limiting factors for marine animals. 3. Relative resistance to high temperatures." *Contrib. to Canadian Biol.* N.S. **2**, No. 6.

ISSEL, R. (1925). "Contributo alla conoscenza ecologica delle larve planctoniche di *Cefalopodi.*" *R. Comit. Talass. Ital. Mem.* **120**.

JESPERSEN, P. (1925). "On the quantity of macroplankton in the Mediterranean and the Atlantic." *Intern. Rev. Hydrobiol. Hydrogr.* Bd. **12**, Heft 1/2, 102–115.

JESPERSEN, P. and VEDEL TÅNING, Å. (1926). "Mediterranean Sternoptychidae." *Rep. Danish Ocean. Exped.* 1908–10, **2**, No. 9 (*Biology*), A 12.

JOHANSEN, A. C. (1925). "On the diurnal vertical movements of young of some fishes in Danish waters." *Medd. fra Komm. f. Havunders. Serie Fiskeri*, Bd. **7**, Nr 2.

KARSTEN, G. (1905–6–7). "Das Phytoplankton des Antarktischen Meeres nach dem Material der deutschen Tiefsee-Expedition 1898–99. Das Phytoplankton des Atlantischen Oceans, etc. Das Indische Phytoplankton." *Wiss. Ergeb. der deutsch. Tiefsee-Expedition auf dem Dampfer 'Valdivia.'* Zweiter Band, Zweiter Teil. Jena.

KLUGH, A. B. (1925, *a*). "On the effect of light of different wave lengths on the rate of reproduction of *Volvox aureus* and *Closterium acerosum.*" *The New Phytologist*, **24**, No. 3, 186–190.

—— (1925, *b*). "Ecological photometry and a new instrument for measuring light." *Ecology*, **6**, No. 3, 203.

—— (1927). "Light penetration into the Bay of Fundy and into Chamcook Lake, New Brunswick." *Ecology*, **8**, No. 1, 90–93.

KNIEP, H. and MINDER, F. (1909). "Ueber den Einfluss verschiedenfarbigen Lichtes auf die Kohlensäureassimilation." *Zeit. f. Bot.* **1**, 619–650.

KNIPOWITSCH, N. M. (1926). "Zur Hydrologie und Hydrobiologie des Schwarzen und des Asowschen Meeres." *Intern. Rev. Hydrobiol. Hydrogr.* Bd. **16**, 81–102.

KNUDSEN, M. (1922). "On measurement of the penetration of light into the sea." *Cons. Perm. Intern. pour l'Explor. de la Mer. Publ. de Circonstance*, No. 76, 1–16.

KRAEFFT, F. (1910). "Über das Plankton in Ost- und Nordsee und den Verbindungsgebieten, mit besonderer Berücksichtigung der Copepoden." *Wissen. Meeresunter.* Kiel, **11**.

KRAMP, P. L. (1915). "Medusae, Ctenophora and Chaetognatha from the Great Belt and the Kattegat in 1909." *Medd. fra Komm. for Havunders. Serie Plankton*, Bd. **1**, No. 12.

KRÜMMEL, O. (1893). "Geophysikalische Beobachtungen." *Ergeb. der Plankton-Expedition der Humboldt-Stiftung*, Bd. **1** c.

LO BIANCO, S. (1903–4). "Le pesche abissali eseguite da F. A. Krupp col Yacht Puritan nelle adiacenze di Capri ed in altre località del Mediterraneo." *Mittheil. Zool. Stat. Neapel*, **16**, 109.

—— (1909). "Notizie biologiche riguardanti specialmente il periodo di maturità sessuale degli animali del golfo di Napoli." *Mittheil. Zool. Stat. Neapel*, **19**, 513–761.

LOEB, J. (1893 *a*). "Ueber künstliche Umwandlung positiv heliotropischer Tiere in negativ heliotropische und umgekehrt." *Arch. ges. Physiol.* **54**, 81–107.

—— (1893, *b*). "On the influence of light on the periodical depth-migrations of pelagic animals." *Bull. U.S. Fish. Comm.* **13**, 65–68.

—— (1906). "Ueber die Erregung von positivem Heliotropismus durch Säure, insbesondere Kohlensäure und von negativem Heliotropismus durch ultraviolette Strahlen." *Arch. ges. Physiol.* **115**, 564–581.

—— (1908). "Über Heliotropismus und die periodischen Tiefenbewegungen pelagischer Tiere." *Biol. Centralb.* Bd. **28**, 732–736.

LOHMANN, H. (1902). "Die Coccolithophoridae." *Arch. für Protistenkunde*, Bd. **1**, 89–165.

—— (1908). "Untersuchungen zur Feststellung des vollständigen Gehaltes des Meeres an Plankton." *Wissen. Meeresunter.* Kiel, N.F. Bd. **10**, 131–370.

—— (1909–10). "Die Strömungen in der Strasse von Messina und die Verteilung des Planktons in derselben." *Intern. Rev. Hydrobiol. Hydrogr.* Bd. **2**, 505–556, and Bd. **3**, 275–298.

—— (1920). "Die Bevölkerung des Ozeans mit Plankton nach den Ergebnissen der Zentrifugenfänge während der Ausreise der 'Deutschland,' 1911. Zugleich ein Beitrag zur Biologie des Atlantischen Ozeans." *Archiv für Biontologie. Gesel. Naturforsch. Freunde, Berlin*, Bd. **4**, Heft 3, 1–617.

MACDONALD, R. (1927). "Food and Habits of *Meganyctiphanes norvegica.*" *Journ. Mar. Biol. Assoc.* N.S. **14**, No. 3, 753–784.

MARSHALL, S. and ORR, A. P. (1927). "The relation of the plankton to some chemical and physical factors in the Clyde Sea Area." *Journ. Mar. Biol. Assoc.* N.S. **14**, No. 4.

MAST, S. O. (1921). "Reactions to light of the larvae of the Ascidians, *Amaroucium constellatum* and *Amaroucium pellucidum*, with special reference to photic orientation." *Journ. Exper. Zool.* **34**, No. 2, 149–187.

MAST, S. O. and GOVER, M. (1922). "Relation between intensity of light and rate of locomotion in *Phacus pleuronectes* and *Euglena gracilis* and its bearing on orientation." *Biol. Bull.* **43**, 203–209.

MATTHEWS, D. J. (1923). "Physical Oceanography," in *A Dictionary of Applied Physics.* Macmillan and Co., Ltd., London, 665–692.

MENKE, H. (1911). "Periodische Bewegungen und ihr Zusammenhang mit Licht- und Stoffwechsel." *Pflüger's Archiv ges. Physiol.* **140**, 37.

MICHAEL, E. L. (1911). "Classification and vertical distribution of the Chaetognatha of the San Diego region." *Univ. Calif. Public. Berkeley*, **8**, No. 3, 21–186.

—— (1913). "Vertical distribution of the Chaetognatha of the San Diego region in relation to the question of isolation versus coincident distribution." *Amer. Naturalist*, **47**, 17–49.

MIELCK, W. (1926). "Die Verbreitung der grosseren Planktontiere in der Ostsee April 1925." *Deutsch. Wiss. Komm. Meeresforsch.* N.F. Bd. **2**, 87–91.

MIQUEL, P. (1892). "Recherches expérimentales sur la physiologie, la morphologie et la pathologie des diatomées." *Annales de Micrographie*, **4**.

MOHN, H. (1905). "Meteorology." *Norwegian North Polar Expedition* 1893–1896. *Scientific Results*, **6**, No. 16.

MOORE, A. R. (1912). "Concerning negative phototropism in *Daphnia pulex*." *Journ. Exper. Zool.* **13**, No. 4, 573–575.

MOORE, B. (1909). "Reactions of marine organisms in relation to light and phosphorescence." *Trans. Liverp. Biol. Soc.* **23**, 19.

MURRAY, J. and HJORT, J. (1912). *The Depths of the Ocean.* Macmillan and Co.

NIKITIN, V. (1926). La distribution verticale du plancton dans la mer Noire. I. Copepoda et Cladocera. *Acad. Sci. Républ. Soviét. Social.* Ser. II, No. 9, 93–140. Travaux du laboratoire zoologique et de la station biologique de Sébastopol. (In Russian, with a French summary.)

OBERWIMMER, A. (1898). "Mollusken. II. Heteropoden und Pteropoden, Sinusigera, gesammelt von S.M. Schiff 'Pola,' 1890–1894." Zool. Ergeb. x. *Densk. d. kais. Ak. d. Wiss. Wien*, Bd. **65**, 573–595.

OSTENFELD, C. H. (1913). "De Danske Farvandes Plankton. I. Aarene 1898–1901. Phytoplankton og Protozoa. I. Phytoplanktonets livskaar og biologi, samt de i vore farvande agtagne phytoplanktonters optraeden og forekomst." *D. Kgl. Dansk. Selsk. Skrifter*, 7 Raekke, Naturvidensk. og Mathem. Afd. **9**, No. 2.

OSTWALD, W. (1902). "Zur Theorie des Planktons." *Biol. Centralb.* Bd. **22**.

OTTERSTROM, A. (1910). "Beobachtungen über die senkrechten Wanderungen des Mysis-bestandes in der Ostsee bei Bornholm in den Sommermonaten 1906 und 1907." *Medd. Komm. Havunders. Plankton*, **1**, No. 9.

PALITZSCH, S. (1911–13). "Sur le mesurage et la grandeur de la concentration en ions hydrogène de l'eau salée." *Comptes-rendus Laborat. Carlsberg*, **10**, 85–98.

PARKER, G. H. (1902). "The reactions of Copepods to various stimuli, and the bearing of this on daily depth migrations." *Bull. U.S. Fish. Comm.* 1901, **21**, 103–123.

PAVILLARD, J. (1926). "Bacillariales." *Rep. Danish Ocean. Exped.* 1908–1910, **2**, No. 9 (Biology), J. 4, pp. 1–72.

POOLE, H. H. (1925). "On the photo-electric measurement of submarine illumination." *Sci. Proc. Roy. Soc. Dublin*, **18**, 99–115.

POOLE, H. H. and ATKINS, W. R. G. (1926). "On the penetration of light into sea water." *Journ. Mar. Biol. Assoc.* N.S. **14**, No. 1, 177–198.

REGNARD, P. (1891). *Recherches expérimentales sur les conditions physiques de la vie dans les eaux.* Paris: G. Masson, 1891.

REMOTTI, E. (1921). "Variazioni di peso specifico nelle uova galleggianti dei Teleostei durante lo sviluppo." *R. Comit. Talass. Ital. Mem.* **80**.

—— (1926). "Fenomeni fotodinamici in alcune forme embrionali a dominio luminoso variabile." *Bollet. Pesca. Piscicult. e Idrobiol.* **2**, 42–54.

RITTER ZÁHONY, R. (1909). "Chätognathen." *Zoolog. Ergebn. d. Exped. S.M. 'Pola' in das östliche Mittelmeer*, 1890–94, **14**, 1–18.

—— (1910). "Chaetognatha from the coasts of Ireland." *Fisheries, Ireland, Sci. Invest.* No. 4, 1–7.

ROBERT, H. (1922). "Notes critiques de méthodologie. L'emploi du filet et de la pompe dans les pêches de plancton." *Ann. de Biol. Lacustre*, Tome **11**, Fasc. 1, p. 208.

RÖMER, F. (1904). "Die Ctenophoren." *Fauna Arctica*, Bd. **3**, Lief. 1, pp. 65–90.

ROSE, M. (1923). "Recherches biologiques sur le Plankton." *Bull. l'Inst. Océan. Monaco*, No. 425, 1–8.

—— (1924). "Recherches biologiques sur le Plankton." *Bull. l'Inst. Océan. Monaco*, No. 439, 1–6.

—— (1924). "Action du *p*H extérieur sur le phototropisme des Copépodes pélagiques marins." *Arch. Physique Biologique*, **3**, No. 2, 33–41.

—— (1925). "Contribution à l'étude de la biologie du plankton; le problème des migrations verticales journalières." *Archiv. Zool. Expér. Génér.* **64**, Fasc. 5, 387–542.

—— (1926). "Le plankton et ses relations avec la température, la salinité et la profondeur." *Ann. l'Inst. Océan.* N.S. **3**, Fasc. 4, 161–242.

RUSSELL, F. S. (1925). "The vertical distribution of marine macroplankton. An observation on diurnal changes." *Journ. Mar. Biol. Assoc.* N.S. **13**, No. 4, 769–809.

—— (1926, *a*). "The vertical distribution of marine macroplankton. II. The pelagic young of teleostean fishes in the daytime in the Plymouth area, with a note on the eggs of certain species." *Journ. Mar. Biol. Assoc.* N.S. **14**, No. 1, 101–159.

—— (1926, *b*). "The vertical distribution of marine macroplankton. III. Diurnal observations on the pelagic young of teleostean fishes in the Plymouth area. *Journ. Mar. Biol. Assoc.* N.S. **14**, No. 2, 387–414.

—— (1926, *c*). "The vertical distribution of marine macroplankton. IV. The apparent importance of light intensity as a controlling factor in the behaviour of certain species in the Plymouth area." *Journ. Mar. Biol. Assoc.* N.S. **14**, No. 2, 415–440.

—— (1927). "The vertical distribution of marine macroplankton. V. The distribution of animals caught in the ring-trawl in the daytime in the Plymouth area." *Journ. Mar. Biol. Assoc.* N.S. **14**, No. 3, 557–608.

RUUD, B. (1926). "Quantitative investigations of plankton at Lofoten, March–April, 1922–1924. Preliminary Report." *Rep. Norweg. Fish. Mar. Invest.* **3**, No. 7, 3–30.

SANDSTRÖM, W. J. (1918). "The hydrodynamics of Canadian Atlantic waters." *Canadian Fisheries Expedition*, 1914–15, 221–343.

SANTUCCI, R. (1925). "Contributo allo studio dello sviluppo postembrionale degli 'Scyllaridea' del Mediterraneo. II. *Scyllarus arctus* (L.). III. *Scyllarides latus* Latr." *R. Comit. Talass. Ital. Mem.* **121**.

SAVAGE, R. E. (1926). "The plankton of a herring ground." *Min. Agric. Fish. Fishery Investigat.* Ser. 2, **9**, No. 1.

SCHEWIAKOFF, W. (1926). "Acantharia. Fauna e Flora del Golfo di Napoli." *Pubbl. Staz. Zool. Napoli.*

SCHMIDT, J. (1925). "On the contents of oxygen in the ocean on both sides of the Panama." *Science*, **61**, No. 1588, 592.

—— (1906). "Contributions to the life-history of the eel (*Anguilla vulgaris*)." *Cons. Perm. Intern. pour l'Explor. de la Mer. Rapp. et Proc. Verb.* **5**, 137.

SCHMIDTLEIN, R. (1879). "Vergleichende Uebersicht uber das Erscheinen grösserer pelagischer Thiere während der Jahre 1875–77." *Mittheil. Zool. Stat. Neapel*, **1**, 119–123.

—— (1881). "Vergleichende Übersicht uber das Erscheinen grosserer pelagischer Thiere und Bemerkungen uber Fortpflanzungsverhältnisse einiger Seethiere im Aquarium." *Mittheil. Zool. Stat. Neapel*, **2**, 162–175.

SCHOULEJKIN, W. (1926). "Optische Eigenschaften des Meereswassers und ihr Einfluss auf die Farbe der Tiefseetiere." *Intern. Rev. Hydrobiol. Hydrogr.* **15**, Heft 1/2, 72–78.

SERNOV, S. A. (1910–11). "Grundzüge der Verbreitung der Tierwelt des Schwarzen Meeres bei Sebastopol. Abteilung II. Plankton. Über die vertikale Verteilung des Planktons im Schwarzen Meer." *Intern. Rev. Hydrobiol. Hydrogr.* Bd. **3**, Heft 3/4, 299–305.

SEYMOUR SEWELL, R. B. (1926). "The Salps of Indian seas." *Records of the Indian Museum*, **28**, Pt. 2.

SHELFORD, V. E. (1916). "Physiological differences between marine animals from different depths." *Puget Sound Mar. Stat. Public.* **1**, No. 14.

SHELFORD, V. E. and GAIL, F. W. (1922). "A study of the light penetration into sea-water made with the Kunz Photo-Electric Cell with particular reference to the distribution of plants." *Puget Sound Mar. Stat. Public.* **3**.

SOUTHERN, R. and GARDINER, A. C. (1926). "Reports from the Limnological Laboratory. I. The seasonal distribution of the Crustacea of the plankton in Lough Derg and the River Shannon." *Fisheries, Ireland, Sci Invest.* **1**, 1–170

STEPHENSEN, K. (1923). "Decapoda-Macrura." *Rep. Dan. Ocean. Exped. Mediterr.* 1908–10, **2**, No. 7 (*Biology*), D. 3, 1–85.

—— (1926). "Hyperiidea-Amphipoda (Pt. 3)." *Rep. Dan. Ocean. Exped. Mediterr.* 1908–10, **2**, No. 9 (*Biology*), D. 5, 151–252.

STEUER, A. (1910). *Planktonkunde.* Leipzig und Berlin.

STEUER, A. (1912–13). "Einige Ergebnisse der VII Terminfahrt S.M.S. Najade im Sommer 1912 in der Adria." *Intern. Rev. Hydrobiol. Hydrogr.* 5, 551–570.

—— (1915). "Horizontale und vertikale Verteilung der Copepoden nach den Ergebnissen der deutschen Tiefsee-Expedition.' *Intern. Rev. Hydrobiol. Hydrogr.* 7, Heft 2/3, 205–213.

STRASBURGER (1912). *A Text-Book of Botany.* Macmillan and Co.

TAYLOR, W. R. (1925–26). "Third report on the marine algae of the Dry Tortugas." *Carnegie Institution Year Book*, No. 25, 255–257.

THOULET, J. (1905). "Mémoires océanographiques. VI. Étude sur la transparence et la couleur des eaux de mer." *Rés. Camp. Sci. Albert 1ere. Monaco*, Fasc. 29, 113–134.

—— (1908). *Instruments et Opérations d'Océanographie Pratique.* Paris.

UCHIDA, T. (1926). "The anatomy and development of a rhizostome Medusa, *Mastigias papua* L. Agassiz, with observations on the phylogeny of Rhyzostomae." *Journ. Facult. Sci. Imper. Univ. Tokyo*, Sec. IV. Zoology, 1, Pt. 1, pp 45–95.

WALTHER, J. (1893). *Allgemeine Meereskunde.* Leipzig.

WEISMANN, A. (1876). "Das Thiereleben im Bodensee." *Schrift. Verein. ges. Bodensee*, Bd. 7.

WHIPPLE, G. C. (1908). *The Microscopy of Drinking Water.* London: Chapman and Hall.

WILLEY, A. (1919). "Report on the Copepoda obtained in the Gulf of St Lawrence and adjacent waters, 1915." *Canadian Fisheries Expedition*, 1914–15, 173–220.

WOLTERECK, R. (1904). "Ueber die Entwicklung der Velella aus einer in der Tiefe vorkommenden Larve. Erste Mittheilung über die Tiefplankton-Fänge der Zoologischen Station in Villefranche s.m." *Zool. Jahrbüch.* Suppl. 7, 347–372.

WULFF, A. (1926). "Die Untersuchungsfahrt des Poseidon in der Ostsee im Frühjahr 1925. 3. Die Untersuchungen über die Menge und Zusammensetzung des Planktons mit der Zentrifugier-Methode." *Deutsch. Wissen. Komm. f. Meeresforsch.* N.F. 2, 71–86.

ON SOME FUNDAMENTAL LAWS OF OVARIAN DYNAMICS

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THE endocrine function of the ovary has been studied during recent years in a very intensive manner. Not only has the endocrine function of the ovary been established beyond any doubt, but besides this the *variations* of this ovarian function have been investigated with especial reference to the *rhythm* of this function. The work of Leo Loeb, Stockard and Papanicolaou, Allen, Long and Evans, Courrier, and Marshall and Hammond is well known to every one in this field. The problem of the localization, or of the tissues in which the formation of the ovarian hormones is localized, has also attracted the interest of many workers. Lately the problem of the chemical isolation of the ovarian hormones has entered into a new and brilliant period of development, thanks to the work of Allen and Doisy, of Courrier, of Frank and others.

The influence of the ovarian hormones on morphogenetical processes in the female and their influence on the psycho-sexual behaviour is a very pronounced one; there is, so to say, no tissue in the female which evades this influence. The hormone of the ovary is sex-specific, *i.e.* it is different from the hormone of the testicle, each one being able to stimulate only female or only male sex characters, as has been established by the well-known work of Steinach and confirmed in mammals and birds by different authorities (Sand, Athias, Lipschütz, Krause, Voss and others in mammals; Pézard, Goodale, Zawadowsky in birds). The sex-specificity of the hormones gave a new impulse to an idea first suggested by Tandler, that the sex characters are, so to say, provoked in an *asexual* soma. The author himself was one of those who tried to strengthen this hypothesis. Whether the hypothesis is right or not, the suggestion that there is a "neutral" (Pézard) or an "asexual" embryonic soma which under the influence of female or male sexual hormones develops in the female or in the male direction, gives a clear idea of how profoundly the new data on the sex-specificity of the gonad hormones, and on the dependence of the morphogenetic processes in the organism upon these hormones, have penetrated and influenced scientific thought.

There is still another side to the problem of the endocrine activity of the ovary, namely the problem of *how far the ovarian function depends upon other physiological internal factors outside the ovary*. This problem is by no means a new one. The question of the interrelation of the ovary with other endocrine glands has often

been studied. The dependence of the ovarian function upon the hypophysis is a well-established fact. The influence of food on the ovarian function is also well known, and Hammond, Evans and his associates have studied this influence with most important results. Finally the influence on the endocrine function of the ovary in diseases not directly affecting the latter is well known to the medical practitioner.

During the last six years when studying the internal secretion of the ovary we have learned a series of new facts which make it possible to establish what could be termed "fundamental laws of ovarian dynamics" which might serve as a good basis for further investigation.

The experiments were made during my stay in Dorpat (Tartu) in the University of Esthonia, in collaboration with various associates. The experimental material was published in 1925 and 1926 in *Pflüger's Archiv für die gesamte Physiologie*, vols. 207, 208 and 209, and preliminary communications were made in 1924–1926 in *Comptes rendus de la Société de Biologie de Paris*, vols. 90–93. The laws that I shall propound in this article have been previously discussed in communications made before the Medical Societies of Dorpat-Tartu in Esthonia [1], Riga in Lettland, and Santiago de Chile [2], and a summary has been given in the Russian *Endocrinological Review*.

I. THE LAW OF FOLLICULAR CONSTANCY

It was shown by previous investigators and confirmed by ourselves that the ovary which remains after unilateral castration undergoes a marked hypertrophy [3]. This is due especially to a compensatory follicular development. Arai [4], in the Wistar Institute, showed in the rat that the number of follicles at different stages of development in the one remaining ovary is equal to the respective number in two normal ovaries. This demonstrates that the number of primary follicles which enter into follicular development does not depend upon the total number of primary follicles present in the organism, but rather upon some physiological factors outside the ovary and regulating follicular development. The number of young born after unilateral castration also remains normal. The number of corpora lutea in the ovary after unilateral castration is also normal. These facts have been demonstrated in different animals and lately especially by Hammond and Asdell [5, 6] in the rabbit and by Hartman [7] in the opossum. We stated the same facts as to the number of young born after unilateral castration in the guinea-pig [8].

If the number of primary follicles which enter into follicular development is constant for every species under certain external conditions, and if this number is independent of the total number of primary follicles present in the organism, then an ovarian *fragment* which alone remains in the organism should also be able to produce a normal number of young and a normal number of corpora lutea. The number of new-born after greatly reducing the ovarian mass, and the total number of primary follicles in the organism, have been studied experimentally by Hammond and Asdell [5, 6] in rabbits. They showed that the number of young produced by 1/6 of the total ovarian mass is almost equal to the normal number. As to the

number of corpora lutea in ovarian fragments my own observations on guinea-pigs may be quoted(9). In an ovarian fragment of about 1/3 of one ovary engrafted into a castrated female we found three corpora lutea of the same appearance, *i.e.* of the same age. Pearl and Schoppe(10) had already found in fowls that the number of visible oocytes attains the normal level even in ovarian fragments.

There is another very interesting point as to the behaviour of ovarian fragments. If the number of primary follicles produced by an ovarian fragment remains normal, then it is clear that the total number of primary follicles present in an ovarian fragment will diminish more rapidly than in a corresponding volume of ovarian tissue of normal ovaries. The ovarian fragments should be found a certain time after the operation to be very poor in primary follicles. We have demonstrated this in rabbits and cats(11). In some cases the primary follicles almost disappear, as in one experiment with cats and in several experiments with rabbits. This is true however only in those species where there is no new formation of primary follicles in post-uterine life; it may be different in species where, as shown by Allen(12) for the white mouse, new primary follicles are rhythmically produced.

The behaviour of an ovarian fragment differs from that of normal ovarian tissue not only as to the relative number of primary follicles entering into follicular development, but also as to the further history of the follicle. In most of the experiments with ovarian fragments in rabbits and cats we stated that the ripening follicle shows a tendency to persist and to form follicular cysts(11).

Against the conclusions from experiments with ovarian fragments the objection could be made that the characteristic changes are due, not to some general quantitative factors, but to the operative interference *per se*. Experiments on cats and lately on rabbits (not yet published) have shown us that this objection is not justified. If there are in the organism, besides the ovarian fragment, the remains of the respective ovary and the whole second ovary, in such a manner that the total number of primary follicles is not diminished, then the behaviour of the ovarian fragment does not differ from that of normal ovarian tissue; there is under these circumstances no diminution of primary follicles in the ovarian fragment and there is no formation of big follicular cysts.

The follicular constancy can be demonstrated not only by diminishing the total number of primary follicles by means of unilateral castration or by experiments with ovarian fragments; it can be demonstrated also by *augmenting* the total number of primary follicles. If an ovary is engrafted into a castrated female guinea-pig, then, as already mentioned, there will be a normal number of corpora lutea in the graft. On the contrary, if an ovary is engrafted in a non-castrated female guinea-pig the ovary may take and may undergo follicular development but corpora lutea in similar grafts are never observed(13), whereas in the ovaries *in situ* corpora lutea are always present in these animals. Follicular development evidently stops early in these grafts, hindered by some factor outside the graft[1].

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[1] In one of Marshall and Jolly's(14) experiments with ovarian grafts into normal non-castrated female rats a corpus luteum was found 1½ months after transplantation. We examined our animals about 7 months after operation.

The experiments related above make it clear that the number of primary follicles which enter into follicular development, the degree of follicular ripening which is attained, and the further fate of the follicle depend not upon the total number of primary follicles present, but upon general internal factors outside the ovary. This is what I term the "law of follicular constancy," taking into consideration that there is a constancy for every species.

II. THE LAW OF PUBERTY

Twenty-five years ago C. Foà[15] engrafted embryonic ovaries into adult animals and stated that the follicular development in the graft begins sooner than would correspond to the age of the graft. This statement was confirmed by Tussau[16]. The great significance of these statements was not at first quite appreciated and the significance of it has only become evident in connection with new experimental work. Long and Evans[17] showed in the rat that the ovary of a young female when engrafted into an adult castrated female will cause the appearance of the oestrous changes in the vaginal smear soon after transplantation. In other words, in the adult organism the ovary of a young female will begin its endocrine activity sooner than in the original organism. A similar statement was made by Lipschütz and Voss[18] with ovarian grafts in male animals. Steinach[19] showed that an ovarian graft causes an hypertrophy of the mammary glands and of the teats in a castrated male. The transformation of the mammary apparatus sets in after a certain time of latency, in general after two weeks. Now Lipschütz and Voss used ovaries of guinea-pigs 16 to 17 days old with a weight of 100 gm. The operated males had a weight of 270 to 300 gm. The hypertrophy of the mammary apparatus set in 2 to 3 weeks after operation. In these experiments the ovary began its endocrine activity at an age of about 4 weeks, although normally the first oestrous period appears only at an age of about 8 weeks. In experiments of Miss Adamberg (not yet published) ovaries of new-born guinea-pigs were engrafted into adult castrated females; the vaginal oestrous changes set in here also about 2 weeks afterwards.

From all these experiments it seems easy to conclude that the endocrine activity of the ovary or follicular development by which hormones are produced, is regulated by the "milieu interne" in that sense that the time at which endocrine activity starts, is fixed by this "milieu interne." This idea was first expressed in a very clear manner by Hammond[5][1].

There is an objection which could be made against this conclusion. One might think that the earlier follicular development, as stated by Foà, and that the earlier start of endocrine activity in an ovarian graft from a young female into an adult one, are caused not by the environment of the adult, but by the operative interference with the ovary. Different experiments[20] show that this objection is not justified. We engrafted young ovaries from guinea-pigs 22 to 24 days old, weighing 85 and 90 gm., into castrated males of the same litter. The hormone effect on the mammary

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[1] "The age of puberty is determined by the nutritive state of the soma of the animal and not by age changes in the ovary itself" (p. 27).

glands set in 6 weeks later instead of 2 to 3 weeks later as with grafts into adult males. In other experiments (Figs. 1 and 2) ovaries of adult females were engrafted

(a)   (b)
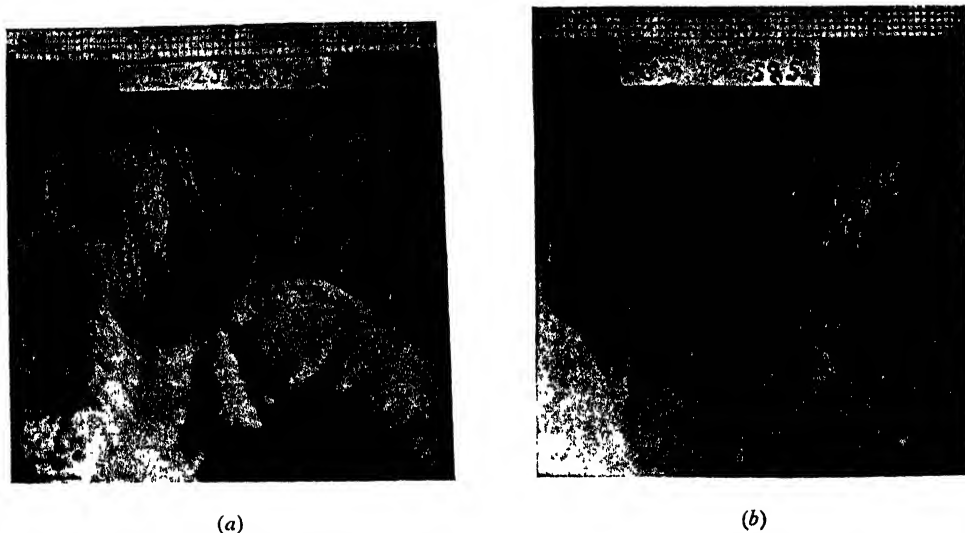
Fig. 1. Transformation of teats in a castrated male which at an age of 96 days and at a weight of 310 gm. was engrafted with ½ an ovary into the kidney. The ovary was that of an adult female weighing 550 gm. operated the 18th of March 1925. Natural size.

(a)   (b)
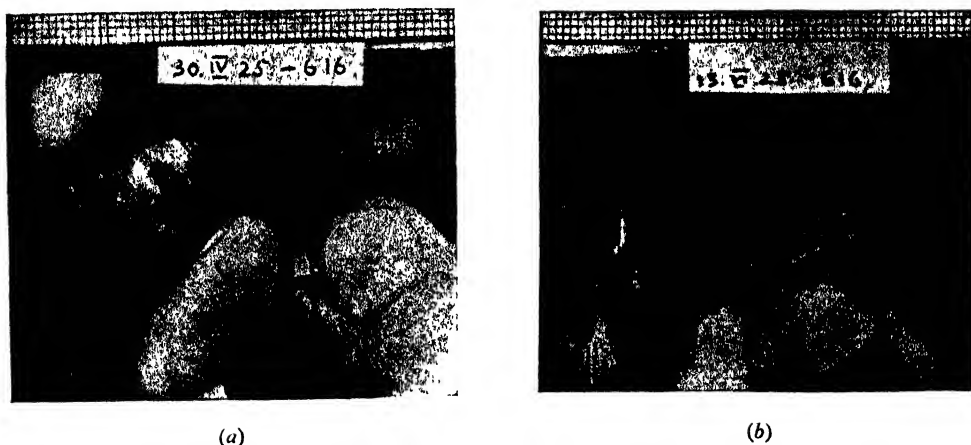
Fig. 2. Condition of the teats in a castrated male which at an age of 34 days and at a weight of 110 gm. was engrafted with ½ an ovary of the same female as in Fig. 1. Natural size.

In 1 (a) the teats are highly hypertrophied whereas at the same time (6 weeks after the operation) the teats were quite rudimentary in 2 (a) though both were engrafted simultaneously with ovary of the same female. Later on the teats in the young animal also hypertrophied [2 (b)]. See also the infantile condition of the penis in 2 (a) and 2 (b), which corresponds to that of an animal castrated at a young age.

into young and adult males. The ovary of an adult female caused a hormone effect 3 weeks after the operation in a castrated male 96 days old; the second ovary of the

same female only caused the endocrine effect 6 weeks after the operation on a castrated male 34 days old.

The above statements have recently been confirmed by Wiesner (21).

From all these experiments it becomes evident that the time at which endocrine activity of the ovary sets in is dependent upon the age of the internal environment and that it is independent of the age of the ovary. When engrafted into an adult animal, the young ovary will begin its endocrine activity sooner than corresponds to the age of the ovary; when engrafted into a young animal, the adult ovary will cease endocrine activity for several weeks. In other words, there is no doubt that the time at which puberty sets in is determined not by the ovary but by the "milieu interne." The ovary is only a means by which sexual puberty is realized when certain internal environmental factors allow follicular development and endocrine activity (the "law of puberty") (22).

III. GROWTH SUBSTANCES

Now the question arises as to what factors are responsible for the different ways in which young and adult animals react to ovarian transplantation. Why is it that the endocrine effect in the young sets in several weeks later than in the adult? At first sight one might think that the tissues of the young are not capable of reacting to ovarian hormones. But there are many facts contrary to this suggestion. In man it has been stated that the mammary apparatus of the new-born child is often more developed than afterwards, in such a manner that the gland of the new-born can even produce colostrum. In new-born guinea-pigs the teats seem more developed than several weeks later. Evidently ovarian hormones influence not only the mammary apparatus of the mother but also that of the offspring. It can also be shown by experiment that the tissues of the young individual react to ovarian hormones with the same rapidity as do those of the adult. Courrier (23) injected follicular fluid into a pregnant guinea-pig 4 days before birth; vaginal changes characteristic for oestrus were observed in the new-born. Allen and Doisy (24) injected follicular fluid into rats 26 days old, and 2 to 3 days later they observed the vaginal changes characteristic of oestrus.

Recently Champy (25) published experiments on frogs in which testicles of young animals were engrafted into castrated adults. Champy stated that the finger callosities of the adult developed under the influence of the young testicle in the same manner as they did after transplantation of adult testicles. On the contrary, there was no development of the pads when testicles of young animals were engrafted into young castrates. Champy thinks that the tissues of the young, unlike those of the adult, are unable to react to testicular hormones; there were in these experiments, according to Champy, hormones, but there was no reaction of the tissues. Our opinion is that this argumentation of Champy is not justified; Champy's supposition, that in the young sex gland which is engrafted into the young animal there are sexual hormones, is in contradiction to what is known of the mammalian ovary. In the mammalian ovary hormone production certainly depends upon follicular development and there can scarcely be any doubt that

there are no hormones in the young ovary which reveals no follicular development. The hormones responsible for the oestrous changes are produced by follicles which have entered into rapid follicular development only a few days before oestrous changes begin, and the fact that there is no endocrine effect for about 6 weeks in the young animal, even when an adult ovary is engrafted, can be explained only by supposing that during all this time no ovarian hormones or only insufficient quantities to cause an endocrine effect are as yet present.

Vintemberger[25a] examined the condition of the mammary glands in young and in adult rabbits injected with follicular fluid. He states that there is a quantitative difference as to the reaction of the mammary gland according to age. I do not feel quite sure whether the observations of Vintemberger are sufficient to draw the conclusion that there really was such a difference.

Here the question arises as to why follicular development and hormone production is not possible in the young. Heape[26], Sand[27, 27a], and Hammond and Marshall[28] suggested that the development of the sex glands depends upon some substances which are present in the blood in limited quantities only. Hammond gave much experimental evidence for this. It would appear that follicular development and hormone production in the young are not yet possible because these X-substances are not yet available and that these substances are produced only when a certain age is attained. Or one might suppose that certain substances necessary for follicular development are required by other organs also, so that there is a competition between the organs and the ovary. Allen and Doisy[24] and Hammond[5] think that the latter may be true. According to Minot[29] the intensity of growth as expressed in the relative increase of weight, gradually decreases; one might then suppose that as age proceeds the quantity of these X- or growth substances available for the ovary increases and that at a certain time the quantity necessary for follicular development is attained.

Under certain experimental conditions the normal balance between the ovary and the body seems to be overthrown, in such a manner that follicular development hinders body-growth. We shall discuss this question in Section IV.

There is much evidence in our experiments that these X-substances or growth substances are the same in both sexes as supposed by Sand. We have seen that the law of puberty can be demonstrated by ovarian grafts both in the female and in the male. In a castrated male which has not yet attained the *age* of puberty (about 150–200 gm.) there is no follicular development; in a castrated male having attained this age follicular development sets in. There is no difference in this respect between male and female. We also gave experimental evidence that these growth substances are uninterruptedly produced in the organism but are immediately used. This is shown by the fact that the time of latency between ovarian transplantation and the beginning of the endocrine effect on the mammary apparatus does not depend on whether testicular castration has been performed just at time of ovarian transplantation or long before[30]. The time of latency seems to vary independently of the time elapsed between testicular castration and ovarian transplantation[1].

[1] All these questions can be studied only on the *castrated* male as otherwise the whole situation

When considering the statements as postulated in Section I (law of follicular constancy) together with the conclusions drawn in Section III, it becomes evident that the same biochemical mechanism might be responsible for the quantitative regulation of follicular development after puberty. The law of follicular constancy and the law of puberty would then both derive from similar interrelations, the one concerning quantitative conditions after puberty, the other quantitative conditions at the time when puberty sets in.

IV. THE SEX-SPECIFIC REACTION TO THE OVARIAN GRAFT

The experiments of Steinach [19] with ovarian transplantation into the castrated male are well known. There was in these experiments not only a development of the mammary apparatus as in the sexual mature female, but there was, as Steinach put it, a "hyperfeminization." The mammary glands and the teats developed as in a pregnant female and there was even production of milk. The statements of Steinach have been confirmed by several investigators, by Sand, by Athias, by Lipschütz and his associates Krause and Voss, and recently by Pettinari.

When observing this striking phenomenon which is so easy to reproduce, the question arises whether the female also will reveal hyperfeminization when engrafted with an ovary. This question is of high theoretical interest. If there were differences between male and female as to the condition of the mammary apparatus after ovarian transplantation, this would be in favour of the conception that follicular development and endocrine activity of the ovary depend upon internal environmental factors, as already discussed in connection with the law of follicular constancy and the law of puberty. Comparative experiments have shown that such differences exist [31, 32].

If an ovary is engrafted into a castrated male and the second ovary of the same female into a castrated female, differences between the two cases become evident several weeks after the operation. Two to three weeks after operation the feminized male reveals the changes characteristic for heat in a young normal female. But instead of regression, which in the normal young female sets in several days later, there is no regression in the feminized male but a progressive development which in about 6 weeks may lead to the condition found in a pregnant or even lactating female. In the female with an ovarian graft there are also, 2 to 3 weeks after operation, the characteristic changes in the teats. They may last longer than in a normal female and the development may proceed further than in the latter. But in the operated female there is, contrary to what has been stated for the feminized male, a certain amount of regression, although not back to the condition of the normal female, the teats remaining always more developed than normally. Later

is interfered with by the inhibiting influence of the testicle. There is no doubt that such an inhibiting influence of the testicles *in situ* exists against the ovarian graft. Only the *mechanism* of this inhibiting influence is still questionable. According to Steinach there is a sex-specific antagonism between testicle and ovary; according to Sand growth substances as discussed above are here in play. Our own experimental observations are rather in favour of there being an action of both these factors. The question of the antagonism of the sex glands is a very complicated one; we have discussed it in various other papers (see especially *Endocrinology*, **9**, 109, 1925; *Pflüger's Arch.* **207**, **208, 211**, 1925–1926).

on the operated female may show new development of the mammary apparatus, and there is a condition like that of the pregnant female as Pettinari (33) insisted upon. But the female does not attain the same degree of hyperfeminization as the feminized male and there is never milk secretion (32). It is possible that the maximal degree of hyperfeminization could be attained in the operated female also, but as yet it has not been observed. Nevertheless the difference between male and female always remains: it is the *rhythm* of the reaction in which this difference is essentially expressed. There is in the feminized male a rapid and uninterrupted development of the mammary apparatus which in most cases is completed about 6 weeks after the operation; there is in the operated female a slow development

(a)

(b)

(c)
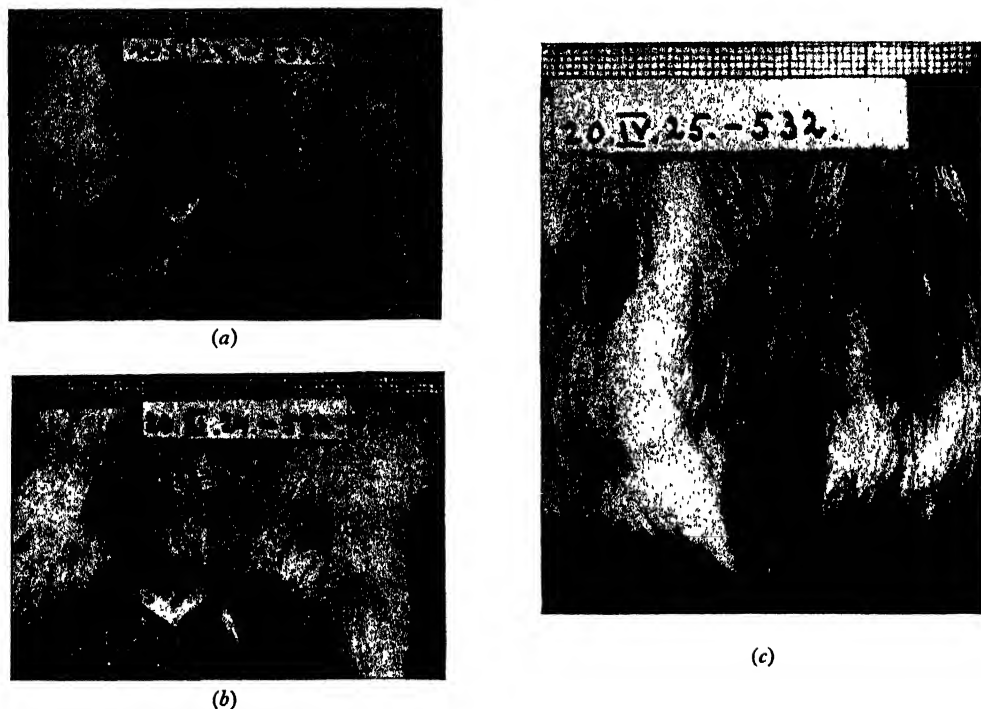
Fig. 3. The teats of a feminized castrated male with ovarian graft. Operation the 1st of November 1924. (a) and (b) ¾ natural size, (c) natural size.

with interruption. Thus if a castrated male and a castrated female which received ovaries from one and the same female are compared, the mammary apparatus of the feminized male will be more developed than that of the operated female at a given moment. It is necessary to insist on this difference. At the beginning we thought (31) that the difference between female and male was greater than here described, and Pettinari (33) objected that the difference between male and female is only a "gradual" one and not an "essential" one. But what do gradual and essential mean? Where is the limit between the two? The fact is that the castrated male and castrated female behave differently when engrafted each with an ovary
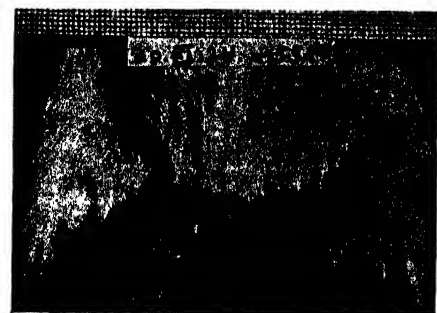
(a)

(b)

(c)

Fig. 4. The teats of a castrated female engrafted with the ovary of the same female as in Fig. 3.

The difference between the male and female can be already observed 2 to 3 weeks after the operation; compare 3 (b) and 4 (b). The difference is still visible 5½ months after the operation. Compare 3 (c) and 4 (c).

(a)

(b)

Fig. 5. Section through mammary gland of male and female engrafted with ovary of the same female. 5½ months after the operation (the same animals as in Figs. 3 and 4). Augmentation about 25. There is a compact glandular tissue in the male, whereas in the female the glandular tissue though present is less compact.

from the same female. It is true that we made our observations on the guinea-pig only, and it cannot be said how far our statements can be generalized. But for the guinea-pig we can speak with surety of a sex-specific reaction to ovarian transplantation (Figs. 3, 4, 5 and 6).

Here the question arises as to whether this difference depends upon somatic differences so that the *tissues* of the male and female react in a different way to ovarian hormones, or whether the *ovarian graft* behaves differently in male and female. The question can be considered as settled in the second sense. The condition of the ovarian graft, according to the sex of the host, has been studied by different investigators. All insist upon differences revealed by histological examinations of the graft. Steinach (19) already knew that certain differences exist. Sand (27)
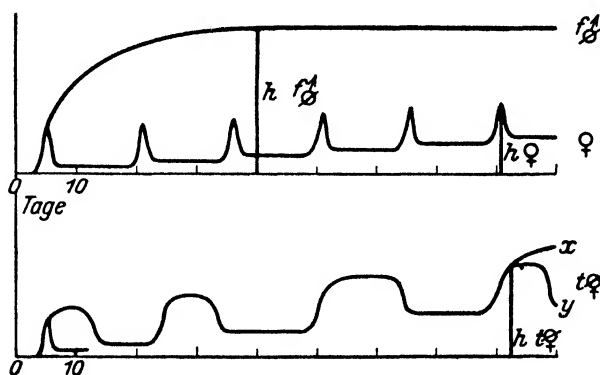
Fig. 6. Diagram of the sex-specific behaviour of the teats in the castrated male and female with testicular grafts. The teat increases progressively in the normal female from one oestrus to another until the maximal size (*h*) in the virgin female is attained. There is during the dioestrum a certain regression. The hypertrophy of the teats in the feminized male (*f* ♂) begins as in the oestrus of the normal female, but there is no dioestral regression, only uninterrupted development until a condition as in the pregnant or lactating female is attained. In the female (*t* ♀) with an ovarian graft the oestrous changes in the teats last sometimes longer than in the normal female and a greater development than in the virgin female can be attained; but there are regressions, and though a condition like that in the pregnant female can be attained, the development is less than in the feminized male. The possibility is not excluded however that in certain cases (*x*), should the experiment have lasted longer, the maximal development as in the lactating female might have been attained.

was the first to examine this question in comparative experiments in male and female rats and guinea-pigs in a detailed manner, and he showed that there is in the female a tendency to the formation of corpora lutea, and in the male a tendency to follicular atresia and not to corpora lutea. Moore (34) and Athias (35) also failed to observe corpora lutea in the male guinea-pig. Only Pettinari (36) claimed to have found them in the male guinea-pig, but he also recognized that follicular atresia and failure to form corpora lutea is the rule in the male. My own observations (9) with ovarian grafts, all of which were made into the kidney (like those of Marshall and Jolly (14) about 20 years ago), fully corroborate these statements.

But there is still another point of great importance in this connection. Athias (35) attracted attention to the fact that the ovarian graft in the male shows a tendency

to *cystic* degeneration; this is also to be seen in Sand's pictures. In my own experiments with intrarenal ovarian grafts (32) it became clearly evident that an ovarian graft which causes hyperfeminization in the male *always* contains large mature follicles with a tendency to form cysts. I do not remember any case of hyperfeminization which was in contradiction to this (Fig. 7).

But what is the meaning of this statement? It can be understood only when the statement is compared with the important findings made by various American investigators in the guinea-pig, the rat and the mouse concerning the coincidence of the ovarian and vaginal cycle. From the work of Long and Evans (17) and of Allen (37) it follows that the progressive phase of the oestrous cycle coincides with the developed Graafian follicle ready to rupture, and that the regressive phase

Fig. 7. Intrarenal ovarian graft into female. About 6 months after transplantation (homoiotransplantation). Augmentation 27; 2 corpora lutea. Several small follicles. Compare Fig. 6.
    The female was a castrated one.

begins with follicular rupturing and formation of the corpora lutea. In other words: in the hyperfeminized male guinea-pig we encounter the ovary in a condition characteristic for the progressive phase of heat. *Hyperfeminization is nothing else than a continued oestrus*. The rapid and uninterrupted development of the mammary apparatus in the engrafted male is caused by an uninterrupted oestrous condition of the ovary. On the contrary, in the engrafted female there is no persistence of ripe follicles or of cysts but rupture and formation of corpora lutea. In accordance with this there is no uninterrupted oestrus in the female with an ovarian graft. There might be here also an irregular endocrine function of the graft, but as in the normal female there are regressions which, as shown by Adamberg (not yet published), may even be of a much longer duration than in the normal animal.

It might be mentioned here that a similar condition as in the hyperfeminized male can be observed in animals with "nymphomania." Recently Courrier (38) has observed a similar condition in a female guinea-pig which, without being pregnant, revealed milk secretion. The histological examination of the ovary showed that there was a cystic degeneration. Similar phenomena can also be observed in the mouse (Allen). On the other hand there is also experimental evidence that protracted injection of follicular fluid causes protracted vaginal changes and mammary development (Allen (39), Ancel and Vintemberger (25a, 40), Courrier (41), Brouha and Simonnet (42), Tuisk (unpublished)).

There can be no doubt that the different reaction of the male and female after ovarian transplantation depends upon the different condition of the ovarian graft in both. We must suppose that there are internal factors responsible for this difference. We have no knowledge as yet as to the nature of these sex-specific factors. They might be localized in some gland of internal secretion; there might be some *sex-specific Y-substances* responsible for this difference and produced by some endocrine gland.

In Section III we have discussed the influence which body-growth may have on the ovary; some *X-substances which are not sex-specific*, and which are necessary both for body-growth and for follicular development, are not yet available for the latter as long as there is an intensive growth of organs before puberty. The experiments with hyperfeminization as related in this section give new support to this idea. Steinach (19) stated for rats and guinea-pigs that the body-weight of the male here resembled that of the female. Sand (27) and Moore (34) failed to corroborate these statements of Steinach, and I myself was rather sceptical. But lately Wang, Richter and Guttmacher (53) have fully confirmed the statements of Steinach; they found that in 12 out of 24 feminized rats the curve of growth was the characteristic one for the female. In some cases they again removed the ovarian graft and almost immediately the curve of body-weight changed and the animals greatly increased in weight. Finally my own experiments convinced me that Steinach was right. I (22) compared the body-weight of feminized guinea-pigs operated at different ages. There was no influence of the ovarian graft on the body-weight when the operated males were already adult or when they had just passed the age of puberty. On the contrary, in those cases where the animals were operated upon very early, at an age of a few weeks only, the body-growth was influenced by the ovarian graft; out of four positive cases two were extremely striking. Now if hyperfeminization is caused by a continuous oestrous condition of the ovary, then one might suppose that the X-substances necessary both for follicular development and for body-growth were in these experiments used up by the ovary and were not available for body-growth, in such a way that body-growth was hindered by the protracted oestrous condition of the ovary. The normal balance between the two is overthrown, as stated above. Hammond (5) suggested that follicular development is hindered during lactation because the mammary gland uses the same X-substances as are necessary for follicular development. Now, in the hyperfeminized male there is not only persistence of a ripe follicle but also persistence of a more or

less active mammary gland. Possibly this coincidence of experimental conditions explains why the normal prepubertal balance between ovary and body-growth is overthrown. Steinach supposed that the influence of the ovarian graft on body-weight could be explained by sex-specific hormones; our explanation seems to be no less correct.

When Steinach discovered the fact that the sex characters of one sex can be promoted only by the gonad of the same sex (sex specificity of the hormones) and that the gonad is able to promote by its hormones the corresponding sex characters in the body of the opposite sex, this was thought by Steinach (19) to be highly in favour of the hypothesis that the somatic substratum is sexually indifferent or identical in both sexes. A similar point of view had previously been held by Tandler (43) on account of his castration experiments in men and mammals, and by Pézard (44) and Goodale (45) on account of their castration and transplantation experiments in fowls. New knowledge about the sex specificity of the hormones was added by Guyénot and Ponse for toads; Ponse (46) observed development of finger callosities in castrated female toads engrafted with testicles, and Welti (47) has confirmed these statements of Ponse. There can be no doubt that the ovary and the testicle really produce sex-specific hormones as Steinach showed for mammals. In view of all this it is really of striking interest to see that an analysis of the same experiments on hyperfeminization which seemed to Steinach, and later on to myself, to be the real basis for the theory of the asexuality or of the indifference of the somatic substratum, reveals to us that the hyperfeminization is not a sign of identity but of difference between female and male in mammals! Certainly, the substratum of the mammary apparatus seems to be indifferent or identical in male and female and its sensitiveness to ovarian hormones is no less identical. Both react with hyperfeminization if the threshold concentration of ovarian hormones is present in the body for a sufficiently long time. In this respect there is no difference between male and female. If possibly not "identical," the somatic substratum is, to use the expression of Zawadowsky (48), "equipotential" in both sexes. But as the ovarian graft behaves differently in both sexes, it becomes clear that some physiological sex-specific factors are present in the body which influence the ovarian graft.

At first sight one might think that the condition of the graft depends upon the presence or absence of the uterus. Leo Loeb (49) finds that the oestrous cycle is prolonged in the guinea-pig if the uterus is removed; corpora lutea remain present for a long time. On the contrary Long and Evans (17) in the rat and Hartman (50) in the opossum did not find any influence of the removal of the uterus on the sexual cycle. It is difficult to explain the difference in the results of the experiments of Loeb and the other authorities. It is conceivable that the condition is different according to the species. There are many observations rather in favour of this suggestion. The rôle of the corpora lutea is most probably different in the guinea-pig and in the rabbit (Ancel and Bouin (51), Hammond (5)), in the cow and in woman (Allen and Doisy (52)). It is also of great interest to know that an ovarian graft in the male *rat* may also produce corpora lutea, although rarely (Sand (27),

Moore (34), Wang, Richter and Guttmacher (53)), whereas, as already insisted upon, corpora lutea are not formed in the ovarian graft in the male *guinea-pig*. Blair Bell (54) and Biedl (55) suggested that the whole endocrine system independently of the sex gland is different according to the sex. As transplantation is made into an animal already sexually differentiated it might indeed be that the difference of the endocrine system is caused by a previous influence of sexual hormones. If the latter is the case, then the difference of the endocrine system according to sex would be a sex character ontogenetically *"fixed"* by hormones (56).

According to Finlay (57) and Greenwood (58) ovarian grafts in the fowl also behave differently according to the sex, there being, in the case of an ovarian graft in the young castrated cockerel, a development of testicular tissue which is able to produce active testicular hormones. No such testicular transformation was observed by these workers in ovarian grafts in females. But contrary to Finlay and Greenwood, Caridroit and Pézard (59) stated that active testicular tissue can be produced in ovarian grafts in the hen also. The suggestion of Finlay that the sexual differentiation of the gonad depends upon some specific influence of the embryonic soma seems therefore unjustified. There are sex-specific influences on the gonad, but these influences do not decide whether a gonad will be male or female; they decide only the further fate of the sexually differentiated gonad.

V. THEORETICAL AND PRACTICAL ASPECTS

We have already discussed in the previous sections some theoretical aspects of the law of follicular constancy, the law of puberty, and the sex-specific reaction to the ovarian graft in the guinea-pig. Some others will be discussed here.

In Section III (law of puberty) we stated that an ovarian graft in an adult animal remains in dioestrum for six weeks, whereas the same ovary in its normal environment would have revealed no less than two oestrous cycles during this time. The oestrous phase fails to take place on account of, physiological environmental conditions. On the other hand the experiments discussed in Section IV (sex specificity) show that an ovary engrafted into a castrated male will persist for weeks, months, or even a year and more, in the oestrous phase, as characterized by follicles maturing but not rupturing. These experiments show clearly that physiological factors outside the ovary control follicular development, as demonstrated by various experiments of Hammond and Marshall (28). We do not know by what mechanism this control is realized. Probably again some X-substances are here in play as Hammond and Marshall supposed.

Now the question arises whether the normal ovarian rhythm is also controlled by certain physiological factors outside the ovary (Heape (26)). In those cases where, as in the rabbit, heat and sexual function takes place only at certain times of the year, external factors such as variations of temperature or of the composition of food, as suggested by Hammond, may be in play. But in those species where, as in the guinea-pig, rat, and mouse, the sexual cycle is repeated at certain intervals throughout the whole year, these external factors do not enter into consideration in explaining the ovarian or sexual cycle. Then the question arises whether the

ovarian rhythm, which undoubtedly causes the sexual rhythm, is an intrinsic, autochthon independent ovarian rhythm, or whether there is a rhythm of internal physiological factors on which the ovary depends. According to L. Loeb[49] follicular development is controlled by the corpus luteum in such a manner that the latter hinders the former. The statements of Loeb would be in favour of the suggestion that the ovarian cycle derives from some kind of *autoregulation*. There is indeed as yet no certainty whether the statements of Loeb for the guinea-pig can be generalized, but these experiments together with those of Hammond and Evans on the dependence of the ovarian cycle on different external factors will lead to a better comprehension or to an explanation of the ovarian cycle.

The fact that the number of follicles entering into follicular development and that the duration of the oestrous and dioestrous phases of the ovary depend upon certain internal physiological factors is also of great interest from the point of view of pathology. I should like to insist especially on the following points. In the section on the law of puberty we saw that an ovary previously in normal cyclical activity in the adult animal remains for many weeks inactive in the young into which it has been transplanted, and that it reassumes its follicular and endocrine activity at a given time when certain physiological factors allow it. Under these experimental conditions a *normal* ovary ceases its activity for a certain time, but remains able to reassume it again. On the other hand, in the section on the sex-specific reaction we showed that a normal ovary, which had previously exhibited the normal cycle in the female, enters into a protracted or uninterrupted oestrus in the male. It seems clear that *serious disturbances of the ovarian cycle may be present in the organism although the ovary is normal.* In view of all the manifold disturbances in the sexual sphere in the case of woman, one is often too much inclined to think that the ovary is the only cause. Certainly, the ovary is here in play, but only secondarily and not primarily. Ovarian transplantation will not help much when two normal but inactive ovaries are already present. The third one will remain inactive as the others when the pathological environment of the ovary inhibits follicular development.

There is much discussion to-day upon the isolation of ovarian hormones and their use in pathology. A normal sexual cycle can indeed be provoked in woman by ovarian hormones. But the question is what is the use of restoring sexual function without restoring its normal rhythm? What we need is to restore the normal ovarian rhythm, and this will be obtained by restoring the "milieu interne," rather than by purified ovarian hormones.

When studying the fundamental laws of ovarian dynamics which are of so great a biological interest we penetrate necessarily into the field of Pathology and Therapeutics.

BIBLIOGRAPHY

* =not seen in the original.

(1) LIPSCHÜTZ, A. (1925). *Eesti Arst* (Tartu), No. 5.
(2) —— (1926). *La Clínica* (Santiago de Chile), **3**, 49.
(3) For literature see MARSHALL, F. H. A. (1922). *The Physiology of Reproduction.* 2nd ed. London.
    LIPSCHÜTZ, A. (1924). *The Internal Secretions of the Sex Glands.* Cambridge and Baltimore.
(4) ARAI, H. (1920). *Amer. Journ. Anat.* **27**, 405; **28**, 59.
(5) HAMMOND, J. and MARSHALL, F. H. A. (1925). *Reproduction in the Rabbit.* Edinburgh.
(6) ASDELL, S. A. (1924). *Brit. Journ. Exp. Biol.* **1**, 473.
(7) HARTMAN, C. (1924). *Amer. Journ. Physiol.* **68**, 97.
    —— (1925). *Amer. Journ. Anat.* **35**, 1.
(8) LIPSCHÜTZ, A. et ADAMBERG, L. (1925). *C.R. Soc. Biol.* **93**, 1464.,
(9) LIPSCHÜTZ, A. (1926). *Pflüger's Arch.* **211**, 722.
(10) PEARL, R. and SCHOPPE, W. F. (1921). *Journ. Exp. Zool.* **37**, 101.
(11) LIPSCHÜTZ, A. (1925). *Brit. Journ. Exp. Biol.* **2**, 331.
    LIPSCHÜTZ, A. and VOSS, H. E. V. (1925). *Brit. Journ. Exp. Biol.* **3**, 35.
(12) ALLEN, E. (1923). *Amer. Journ. Anat.* **31**.
    ALLEN, E., KOUNTZ, W. B. and FRANCIS, B. F. (1925). *Amer. Journ. Anat.* **34**, 445.
(13) LIPSCHÜTZ, A., ADAMBERG, L., TIITSO, M. and VEŠNJAKOV, S. (1926). *Pflüger's Arch.* **211**, 682.
(14) MARSHALL, F. H. A. and JOLLY, W. A. (1908). *Quart. Journ. Exp. Physiol.* **1**, 115.
*(15) FOÀ, C. (1901). *Arch. ital. de Biol.* **35**, 364.
*(16) TUSSAU (1922). Quoted from M. Athias, *Libro en honor de Ramon y Cajal*, **2**, 79.
(17) LONG, J. A. and EVANS, H. M. (1922). *Mem. Univ. California*, **6**.
(18) LIPSCHÜTZ, A. and VOSS, H. E. V. (1925). *Pflüger's Arch.* **207**, 583.
(19) STEINACH, E. (1912). *Pflüger's Arch.* **144**, 71.
(20) LIPSCHÜTZ, A. (1925). *C.R. Soc. Biol.* **93**, 1066.
    —— (1926). *Pflüger's Arch.* **211**, 745.
(21) WIESNER, A. (1926). *Abstr. Commun. XIIth Internat. Physiol. Congr.* (*Skand. Arch. f. Physiol.*)
(22) LIPSCHÜTZ, A. (1926). *Journ. de Biol. et Méd. Expér.* (*Moscow*), No. 6, 1.
(23) COURRIER, R. (1924). *Arch. de Biol.* **34**, 369.
(24) ALLEN, E. and DOISY, E. A. (1924). *Amer. Journ. Physiol.* **69**, 577.
(25) CHAMPY, CH. (1925). *C.R. Soc. Biol.* **93**, 1299.
(25 a) VINTEMBERGER, P. (1925). *Arch. de Biol.* **35**, 125.
(26) HEAPE, W. (1905). *Proc. Roy. Soc.* B, **76**, 260.
(27) SAND, K. (1918). *Studier over Kønskarakterer.* Copenhagen.
(27 a) —— (1918). *Pflüger's Arch.* **173**.
(28) HAMMOND, J. and MARSHALL, F. H. A. (1923). *Proc. XIth Internat. Physiol. Congr.* 137.
(29) MINOT, S. (1891). *Journ. Physiol.* **12**.
    —— (1908). *The Problem of Age, Growth and Death.* London.
(30) LIPSCHÜTZ, A. and VOSS, H. E. V. (1924). *C.R. Soc. Biol.* **90**, 1141.
    LIPSCHÜTZ, A. and assoc. (1926). *Pflüger's Arch.* **211**, 697.
(31) LIPSCHÜTZ, A. and TIITSO, M. (1925). *C.R. Soc. Biol.* **92**, 143.
(32) LIPSCHÜTZ, A. (1925). *C.R. Soc. Biol.* **93**, 1463.
    —— (1926). *Pflüger's Arch.* **211**, 697 and 722.
(33) PETTINARI, V. (1925). *C.R. Soc. Biol.* **92**, 1228.
(34) MOORE, C. R. (1920). *Science*, **52**, 179.
    —— (1921). *Journ. Exper. Zool.* **33**, 192 and 365.
(35) ATHIAS, M. (1922). *Libro en honor de D. Santiago Ramon y Cajal.* Madrid.
(36) PETTINARI, V. (1923). *Arch. per le Sc. Med.* **46**, 338.
    —— (1926). *Atti. Soc. Lomb. di Sc. Med. e Biol.* **15**.
(37) ALLEN, E. (1923). *Amer. Journ. Anat.* **30**, 297. (Quoted from ALLEN (1924). *Ibid.* **34**, 133.)
(38) COURRIER, R. (1925). *C.R. Soc. Biol.* **93**, 674.
(39) ALLEN, E. (1924). *Amer. Journ. Anat.* **34**, 133.
(40) ANCEL, P. and VINTEMBERGER (1924). *C.R. de l'Assoc. des Anat. Paris, Edit. Médic.* (Quoted from P. ANCEL et P. BOUIN.)
(41) COURRIER, R. (1926). *C.R. Acad. Sci.* **182**, 1492
(42) BROUHA, L. and SIMONNET, H. (1925). *C.R. Soc. Biol.* **93**, 557.
(43) TANDLER, J. and GROSZ, S. (1913). *Die biologischen Grundlagen der sekundären Geschlechtscharaktere.* Berlin.

(44) PÉZARD, A. (1918). "Le conditionnement physiologique des charact. sex. second. chez les Gallinacés." Ed. de *Bull. Biol. de la France et de la Belgique*. Paris.
—— (1915). *C.R. Acad. Sci.* **158**, 613.
PÉZARD, A., SAND, K. et CARIDROIT, E. (1924). *C.R. Acad. Sci.* **178**, 2011.
(45) GOODALE, H. D. (1916). *Gonadectomy in Relation to the Secondary Sexual Characters of some Domestic Birds. Carnegie Institution Publications*. Washington.
(46) PONSE, K. (1923). *C.R. Soc. de Physiol. et de l'Hist. natur. de Genève*, **40**, 150.
—— (1924). *Revue Suisse de Zool.* **31**, 177.
(47) WELTI, E. (1925). *C.R. Soc. de Physiol. et de l'Hist. natur. de Genève*, **42**, 133.
(48) ZAWADOWSKY, M. M. (1922). *Das Geschlecht und die Entwicklung der Geschlechtsmerkmale* (Russian, with German Summary). Moscow.
(49) LOEB, L. (1910). *Science*. Reviewed in LOEB, L. (1923). *Amer. Journ. Anat.* **32**, 305.
(50) HARTMAN, C. (1925). *Amer. Journ. Anat.* **35**, 25.
(51) ANCEL, P. et BOUIN, P. (1911). *Journ. de Physiol. et de Pathol. génér.* **13**.
—— —— (1924). *C.R. de l'Assoc. des Anat. Paris, Edit. Médic.*
(52) ALLEN, E. and DOISY, A. (1925). *Proc. Soc. Exp. Biol. Med.* **22**, 303.
(53) WANG, G. H., RICHTER, C. P. and GUTTMACHER, A. F. (1925). *Amer. Journ. Physiol.* **73**, 581.
(54) BELL, W. BLAIR (1913). *Lancet*, **1**, 944.
—— (1920). *The Sex-Complex*. 2nd ed. London.
(55) BIEDL, A. (1922). In *Sexualreform und Sexualwissenschaft*, ed. by A. Wiel, Stuttgart, p. 14.
(56) LIPSCHÜTZ, A. (1918). *Arch. f. Entw.-Mech.* **44**, 207.
—— (1919). *Die Pubertätsdruse u. ihre Wirkungen*. Bern.
(57) FINLAY, G. F. (1925). *Brit. Journ. Exp. Biol.* **2**, 439.
(58) GREENWOOD, A. W. (1925). *Brit. Journ. Exp. Biol.* **2**, 469.
(59) CARIDROIT, E. et PÉZARD, A. (1925). *C.R. hebdom. des séances de l'Acad. des Sc.* **180**, 2067.

SOME PROBLEMS IN THE EVOLUTION OF THE ECHINOIDEA

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THE Echinoderma are perhaps better suited for investigation of problems of morphogeny and phylogeny than any other invertebrate phyla, owing to their possession of mesodermal skeletal structures. Within the phylum the Echinoidea are especially favourable subjects of study, for their tests are usually fairly coherent after death, and the range of the Class (from the Ordovician to the present day) keeps within the bounds of fossiliferous horizons, making relatively complete evidence accessible. An attempt is made here to indicate one or two of the aspects of evolution on which study of the Echinoidea seems to throw light; but even these can be touched on but lightly.

PERSISTENCE OF ROOT-STOCKS. *Cidaris* (*sens. lat.*) ranges from the Devonian to the present day, showing a minimum of change. While stocks presumably descended from it have waxed and waned, and produced various highly complex and even bizarre modifications, this beautiful, but generalized, type has persisted through geological and evolutional crises virtually unaltered. *Cidaris* is comparable in this respect with *Lingula* and *Nucula*. On another analogy, if *Bothriocidaris* is the "Adam" of the class, *Cidaris* is its "Noah," for it alone survived the Permo-Triassic debacle which overwhelmed the Perischoechinoids, and it alone begot all the succeeding Euechinoid population. This durability of simple types is illustrated (in lesser degree) by the early members of all Euechinoid Orders—as, for example, the *Hemicidaris* group, the *Holectypus* group and the *Fibularia* group.

As a theoretical rider to these facts, it may be surmised that each founder of a new stock represents the minimum of specialization required for success in a given (or chosen) habitat; and if the conditions selected are constant or widely distributed (such as deep water, beaches, and the like) there is little need for evolutional change and small risk of extinction.

"SCATTER-POINTS." Echinoid evolution, like that of most other groups, does not encourage reference to "genealogical trees" in the usually accepted sense. It might be compared with a plant in which foliage and branches spring out periodically at well-marked nodes, between which mere bract-like offshoots are produced. An outstanding illustration of this spasmodic outburst of specialization is found in the Palaeozoic Perischoechinoidea. Assuming (in conformity with all the evidence) that the class originated in Ordovician times, we find specialization of intense degree

before the close of the Silurian period, while during the Carboniferous period we watch extravagances never afterwards attained by the Class. Again, in late Triassic times, when the sole representative of the Echinoids was a Cidaroid lineage, we meet suddenly with well-established members of the "Diademoids," the Holecty-poids and the Nucleolitoids. Subsequently, in the Cretaceo-Tertiary, when many Mesozoic stocks had failed or were declining, the seas became rapidly populated by Cake-Urchins, Heart-Urchins and the modern Echinidae.

A reasonable corollary to this evidence of the periodic occurrence of evolution of ordinal value would be that absence of competition encourages rapid variation. Lack of competition may result from the disappearance of previous occupants of a certain environment, or from the invasion of a new environment by members of a race hitherto strangers to it. Competition leads to stereotyping, since serious deviation from a type that has proved its worth sufficiently to leave descendants would more often lead to less efficiency than to more. Given a constant environment and a housing problem, variants are at a discount. But with a change of environment (either imposed or chosen) there is, at first, freer scope for variants to make good. This effect can be illustrated very clearly in the early history of land-plants, reptiles and mammals. The struggle for existence prevents (or at least retards) the "origin of species"; absence of competition gives licence for the establishment of new types.

PROGRESSIVE EVOLUTION. The spasmodic specialization discussed in the preceding paragraph is concerned only with modifications of sufficient importance to affect the bionomics of a stock. When minor details of structure which (if effective at all) merely change the quantitative or qualitative efficiency of an adaptive structure are examined, the influence of steady infinitesimal change along definite trends is obvious. Indeed, the late Dr A. W. Rowe and the genus *Micraster* may be said to have given between them the first tangible proof of steady progress in evolution. Other genera too, when traced through deposits which indicate constancy of environment, show comparable piecemeal progress. But the variation involved in this type of evolution leaves vitally important structures almost unchanged, and is restricted to detail of ornament or proportion that often appears (to our ignorance) utterly gratuitous.

In view of the nature of progressive change shown in the *Micraster*-sequence and comparable series, two conclusions seem justified. Firstly, variation has a definite impulse and a definite trend. These are revealed when the disturbing factor of changing environment is eliminated with consequent discouragement of radical change in the inhabitants. Secondly, steadily progressive evolution may lead to more perfect adaptive structures (should such be possible), as in the case of the complexity and strength of Diademoid ambulacra (or in the familiar evolution of horses' feet and teeth); or it may produce modifications that seem quite unrelated to environment.

THE ECHINOID LANTERN. The history of this marvellous mechanism serves to illustrate in morphogenesis the phylogenetic principles proposed above. The lantern consists of 35 separate ossicles formed from at least 40 centres of calcification.

It is found complete in Silurian Echinoids, and there is every indication that it was fully developed in *Bothriocidaris*. While we can trace its gradual disappearance (and incidentally find convincing proof of the principle of ontogenetic recapitulation by its development in the post-larval stages of such edentulous forms as *Echinonëus* and *Apatopygus*), we are unable to watch its appearance. We are confronted suddenly with a *fait accompli* at the very outset. It is argued that an incomplete or imperfect lantern would be worse than useless, so that unless the structure could appear promptly and altogether it would not appear at all. Sudden introduction of an elaborate apparatus may suggest teleology; but a far more conscious teleology would· be implied in the conception of a useless structure generated and then slowly made effective—some degree of efficiency must be developed at once for the structure to be retained and improved. When once the jaws are present, later lineages whose habits do not involve much biting can let the structure deteriorate to any stage of inefficiency (as in the Holectypoida), or others which chew a fresh diet in a new way may slowly modify the proportions of the parts of the structure (as in the Clypeastroida).

It appears that there is a persistent evolutional potential which expresses itself variously according to circumstances. When habits and environment remain constant, competition holds in check all but trifling mutations; change of habits and environment give full scope for more rapid and radical variation that might merit the name of saltation. In phylogeny, if the intrinsic capacity for variation, under restraint of competition, gradually gives origin to species, the same capacity, encouraged by immunity from competition in a new environment, may suddenly give rise to genera and families. In morphogeny, a precisely analogous condition holds good; structures undergo slow and steady improvement or deterioration while their functions remain or after they are lost, but they originate in immediate response to a new opportunity or demand.

A REVIEW OF RECENT DEVELOPMENTS IN HISTOCHEMISTRY[1]

BY MAURICE PARAT

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THE most valuable previous review of Histochemistry is that of A. Prenant (1910)[1]. Ten years after writing this, Prof. A. Prenant gave a series of lectures at the Medical School in Paris on the different aspects of Histology. This formed a sequel to his first contribution. It was summarised and published later (1921) in a short paper[2]. It is noteworthy that in both papers a reference is found to the chapter of Mann's[3] *Physiological Histology*, the title of which, "On the Chemistry of some Tissue Constituents," is in itself a real programme.

The results of this association of Histology and Chemistry must be histological, that is to say, *elective and specific*. They must also be chemical, *i.e.* reproducible *in vitro* and, as far as possible, explicable by an equation (Mann, Ehrlich, Prenant). "Ordinary chemical analysis," said Ellermann (1903), "teaches us generally the properties of substances and their amounts in the tissues. Histochemistry goes one step further in trying to show the minute repartition of these substances." An elective, specific histochemical technique reproducible *in vitro*, explicable by an equation, may however be very bad if its results are not *topographic*, if it is impossible to localise the reaction.

Such are the rigorous principles of Histochemistry. Many investigators have not submitted themselves to these laws and the consequence is an *ensemble* of doubtful microchemical tests such as those of Zacharias (1887) and of Schwartz (1888) on the constitution of the nucleus. On the other hand, the boundaries may perhaps be too strictly delimited. Is it necessary, for example, to consider as non-histochemical certain solubility reactions which are specific by their negative character? Probably not, because in some cases very similar tests are used by chemists. The fats, for instance, are defined by such reactions (solubility in alcohol, ether, acetone, etc.). It is well known that bilirubin is soluble in chloroform while biliverdin and biliporphyrin are not, and that biliverdin alone is soluble in acids; bilirubin, biliverdin and biliporphyrin are soluble in the alcalis.

It is nevertheless the duty of the histologist to approach as nearly as possible to the chemist's precision in technique, and in the interpretation of reactions. As in

[1] This review is a summary of lectures which were delivered in the Department of Comparative Anatomy and Histology in the Winters of 1923-26 to the students in Histological Technique.

the case of the chemist the purity of reagents and the cleanliness of glassware
are of the first importance.

The routine of histochemical technique, according to A. Prenant, is as follows:

(1) Determination of acidity and alkalinity.
(2) Localisation of oxidation-reduction processes.
(3) Distinction between living and non-living substances.
(4) Determination of the chemical constituents of the tissues.

The recent progress of the first two classes is such that these techniques must
be ranged among the most modern physicochemical procedures (measurements
of pH and rH). The third is based, above all, on vital staining, the meaning of
which is far from having received an adequate chemical explanation. I shall discuss
here the fourth class only, viz. the determination of the constituents of tissues;
that is to say: (a) organic compounds, and (b) simple elements and inorganic
compounds.

A. ORGANIC COMPOUNDS.

These are: (a) proteins and their derivatives, and other nitrogenous subtances;
(b) fats; (c) carbohydrates[1].

(a) PROTEINS AND THEIR DERIVATIVES; NITROGENOUS SUBSTANCES.

The chemistry of the constitution of cytoplasmic proteins is in the dark. We
have only an intuition of its great complexity and the histochemist is waiting for
the distant day when it will be possible accurately to distinguish the different
proteins in tissues and cells. Cytoplasmic inclusions are, however, often sufficiently
individualised and condensed to permit of certain reactions: this is the case of
secretory granules among which the most typical are those of the fowl's oviduct,
studied by Turchini [4].

It is well known that some solubility reactions have been tried on proteins.
Reinke (1896) was able to dissolve crystalloids of the interstitial glands of the
testis with 10 per cent. NaCl, and he concluded that these are globulins, not
albumins. It is, chiefly, the technique of digestion (Beale) which has been employed
to distinguish collagen, elastic and reticulated fibres (Stirling, Ewald and Kühne,
Hall, Hoehl).

Among the staining reactions are: Millon's test, the details of which were
modified by Denigès [5][2]; Zacharias' technique (fixation of potassium ferrocyanide
by proteins; formation of Prussian blue by addition of iron perchloride); Overton's,
Poulsen's and more recently Derrien and Turchini's [6] reactions based upon the
adsorption of tannin by proteins; Wurster and Raciborski's method with quinone;
those of Ruhemann-Abderhalden with ninhydrin, of Axenfeld with formic acid
and gold chloride, of Krasser with alloxan, and of Romieu [7] with orthophosphoric
acid.

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[1] I shall omit a discussion of the carbohydrates because nothing new has been added to our
knowledge recently.
[2] This reagent is best adapted for marine organisms and acts at room temperature.

Many other techniques in the literature are useless because the tissues or the cells are changed by too high a temperature or by too concentrated reagents. Such are the Xanthoproteic reaction and the reactions of Piotrovsky-Meyer, Adamkiewicz, Seegen, etc.

The staining reactions of the proteins were recently applied by Giroud[8] to the determination of the constituents of mitochondria. The proteic nature of mito-chondria has been maintained by many cytologists; Cowdry, for example, maintained this, although he was unable to prove the hypothesis by Millon's test. A. Meyer[9] thought that mitochondria were proteic substances related to the nucleins; he demonstrated their fixation by 3 per cent. nitric acid, and by alcohol. A. Giroud has clearly shown that in the intestine of *Ascaris* it is possible to have an *ensemble* of positive specific reactions for proteins; this has put the question beyond doubt. Parat and Painlevé[24] have also obtained some proteic reactions with the mito-chondria of the germ cells of *Helix* and especially intensive reactions with the so-called "dictyosomes" (Perroncito). They rely partly upon this fact in denying the "Golgi" nature of these cell constituents.

Among the derivatives of the proteins, we must mention certain pigments, melanin for example, and also the compounds of carotin (a pigment without nitrogen) with globulins and albumins. This carotin-albumin has been admirably studied by J. Verne[10], to whom I refer the reader. The decomposition products of nucleo-proteins, puric substances, have been studied by Courmont and André, and Ciaccio, whose papers are classical. A number of investigations on puric pigments have also been published by J. Millot[11].

In this connection, one of the most interesting nitrogenous substances is adrenalin. The importance of this substance and of its "chromaffin reaction" is common knowledge. This reaction has permitted Stilling (1890–98) and Kohn (1898–1902) to describe the paraganglionic system, and Mulon (1904) to charac-terise *in vivo* and *in vitro* the presence of Takamine's substance.

J. Verne[12], studying the posterior salivary glands of *Octopus*, was surprised to find this reaction localised in peculiar granules in the cells. The substance, however, was not adrenalin, but tyramine. Thus the chromaffin reaction is not specific. Both tyramine and adrenalin are, however, amines whose aromatic
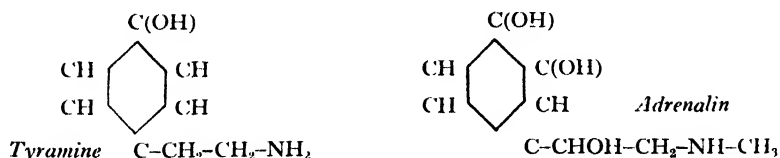
nucleus bears one or two phenolic hydroxyls. Dale and Dixon (1909) had previously compared tyramine with adrenalin. Why do adrenalin and tyramine give the same reaction? Probably because there exists a common group in their formulae which is responsible for the reaction. Verne, investigating different phenols, showed that with pyrocatechine (characteristic grouping: OH–OH ortho), hydroquinone (OH–OH para), pyrogallol (OH–OH–OH 1.2.3), metol [OH–NH (CH₂) para],

adrenalin [OH–OH ortho.NH (CH₃)] the reaction is strong, while it is negative in presence of phenol (OH), risorcine (OH–OH meta), phloroglucine (OH–OH–OH meta), etc. The conclusion is that substances whose nucleus bears two phenolic hydroxyls (ortho or para) or one OH and one NH_2 (one H may be substituted in ortho and para positions) give the reactions; whereas no reaction is obtained when the groupings are in the meta position.

The chromaffin reaction is thus characteristic of organic compounds containing at least two free phenolic groups or one phenol and one amino group in the ortho or para position, except when this group takes part in an amino-acid group. These compounds are rare in animals and are generally toxic.

(b) FATS.

Definition and properties.

The best and most practical definition of the fats is based upon the character of strong solubility in ether, alcohol, chloroform, benzine, and petroleum ether.

Among the fats are classed the true fats, that is to say the glycerol esters constituted by the union of fatty acids with glycerol. Chemically these are well defined. Under the same heading we must range the fatty acids and the soaps, toxic and rare in healthy organisms because in normal conditions they are rapidly saturated by glycerol. Actually, oleic, palmitic and stearic acids are in question.

Related to the true fats we have the lipoids. Histologists have generally conserved the old term "lipoids" because the lack of precision of this word, which is condemned by chemists, is very convenient in the present state of their science. The lipoids may be classified as:

(a) Lipoids without phosphorus – cholesterol.
(b) Lipoids with nitrogen and phosphorus – phosphatids, aminophosphatids.
(c) Carbohydrated lipoids – cerebrosids.

Cholesterol has a very important rôle in organisms because its distribution has a great influence on the hygrophilic state of the cells ("lipocytic coefficient" of Mayer and Schaeffer[1]). Its physical characters are very similar to those of the true fats. By the union of fatty acids with cholesterol there are produced cholesterol esters, with an anisotropic character (cholesterol is isotropic). This might be very important for the histochemist were this character not "masked" by the union of these substances with other lipoids or fats. This is also the reason for which the excellent reactions of Salkowski and Windaus often remain negative. It should be remembered that these reactions may be carried out under a cover-glass on fresh or on fixed tissues cut by the freezing method, using four drops of concentrated sulphuric acid, with one drop of water in the former case, or an alcoholic solution of 0·5 per cent. digitonoside (digitonine) (Brunswick) in the latter. A. Leulier and R. Noël (13) have recently modified this technique by putting the pieces of tissue into a solution of 1 per cent. digitonine in 35 per cent. alcohol

¹ Cf. "Coefficient lipocytique et imbibition des cellules vivantes par l'eau." *C. R. de l'Ac. des Sc.* CLVI, p. 1253, 1913.

for eight days. They cut the pieces with a freezing microtome, observe the sections in polarised light, and are able to characterise the crystals of the digito-cholesterol compound *in situ*. I have in some cases obtained better results by putting the pieces into alcohol with 10 per cent. NaOH for previous saponification.

Among the phosphatids, lecithin is the best known and the most widespread. But Histochemistry is very poor in methods for the detection of this lipoid. Only its anisotropic character and the negative reactions for cholesterol are used for its recognition. Ciaccio has discovered a technique for demonstrating lecithin (*vide infra*), but Fauré-Fremiet, Kawamura, Kaiserling, and Baginski[14] think its specificity must be extended to other substances[1].

Detection of Fats.

What are the different stains and reactions used in Histochemistry for the detection of fats?

According to Fauré-Fremiet, and Mayer and Schaeffer[15], we can distinguish:

I. The specific stains for fats, Sudan III (Daddi) and Scharlach R, whose properties and use are well known.

II. Non-specific stains: quinoleine blue, Nile blue sulphate, neutral red, indophenol blue. Metachromatic changes are frequently observed *in vivo* and on fixed tissues with Nile blue and neutral red. Pellizzi, *in vivo*, and Watrin, on fixed tissues, thought that these changes were characteristic for certain kinds of fats; Fauré-Fremiet[16] has denied the results of Pellizzi, and explained these changes as follows:

Neutral red is strongly dissociated in water, and the aqueous solution includes in consequence:

(*a*) A red salt very soluble in water.

(*b*) An orange-yellow base slightly soluble in water, but very soluble in neutral fats and their solvents.

(*c*) A colourless acid-radical.

Chloroform, benzene or neutral fat in presence of the aqueous solution of neutral red show a yellow colour resulting from a dissolution of the base. A fatty acid, on the contrary, shows a red colour because it has united with the base to form a red soap.

A similar explanation is applicable to Nile blue, with this difference that dissociation of this dye liberates a red base which is more soluble in neutral fats and that the base forms blue soaps with fatty acids (Lorrain Smith). These stains are therefore valuable since they give good indications of the neutrality or acidity of the fats.

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[1] Two years ago we employed for demonstrating lecithin, reactions picked up by Cretin (*Thèse Médecine*, Paris, 1923) from the work of Casanova, based upon the action of sulphuric acid and ammonium molybdate which gives a cherry red, then a blue colour in presence of lecithin, and from the work of Malengrau and Prigent which gives a brown precipitate by action of an alcoholic solution of iron iodide. We are, however, now attempting to improve upon these reactions.

Indophenol blue. The well-known reagent of Köchlin-Witt (1882) (Röhmann and Spitzer, Schultze, 1909) for the demonstration of oxidases in tissues also shows, according to Gräff, fats which are "supra-vitally" stained (*i.e.* stained in living cells separated from the organism). According to J. Zweibaum (17) the reagent may be used immediately after the mixture of α-naphtol + dimethylparaphenylene diamine hydrochloride is prepared ("Nadi" mixture) or later, after the indophenol blue has been formed by oxidation in air[1]. In the first case (Nadi mixture) oxidases are also coloured, but the granules disappear little by little while the fat reaction increases gradually. In the latter case (indophenol blue) the staining of fats is specific and takes place immediately. This reagent may be utilised, of course, on fixed tissues and presents the advantage of acting in the absence of alcohol or other fat solvents.

Zweibaum has vitally stained Protozoa with these reagents, and Zweibaum and Mangenot (18) have applied these reactions to vegetable oils and essences.

III. Osmium tetroxide (OsO_4, osmic acid).

After the introduction of OsO_4, by M. Schultze (1864), most investigators thought they had at hand a specific stain and fixative for fats. Altmann (1894), Unna, Stark, Handwerk, Mulon (19) have shown that it is only a specific reagent for unsaturated fatty acids ($C_nH_{2n-2}O_2$) whose molecules include CH groups (ethylene bond) with a great avidity for oxygen. Among these fatty acids, practically the only interesting member in the animal organism is oleic acid. Mulon has proved that the series of saturated acids ($C_nH_{2n}O_2$) could not be blackened by OsO_4. But a trace only of impurity, that is to say of oleic acid, is sufficient to change a negative reaction to a positive one. This change is more or less intense and depends upon the quantity of oleic acid present in the mixture. When the amount of oleic acid is at least 50 per cent., impure stearic or palmitic acid, for example, is immediately intensely blackened. When the amount is less than 50 per cent. the colour which appears immediately is never black, but brown, grey, or yellow. Subsequent treatment in weak alcohol produces a blackening due to the reducing power of the alcohol ("alcohol-osmium-reduction" of Starke). In the former case (at least 50 per cent.) the fats are well fixed and difficult to dissolve. In the latter case (less than 50 per cent.), on the contrary, they have a great lability.

Osmium tetroxide is thus a useful reagent since it shows the presence of oleic acid, and approximately its amount.

IV. Stains after the fats have been rendered insoluble. Fats may be rendered insoluble by:

(1) Heavy metal salts, for example those of Cu, Hg, Pt, Ur, Cd. The fatty acids are transformed into insoluble or very slightly soluble soaps. This is the principle of the Weigert and of the Benda techniques for fatty acids: fixation in the latter case by formol copper acetate, 2–4 days; the crystallised fatty acids are

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[1] It is interesting to note that the commercial indophenol blue is soluble only in alcohol; on the contrary, nascent indophenol blue is soluble in water and acts best as a "supra vital" stain for the fats 12 hours after its preparation.

green. It is also the principle of Fischler's technique for fatty acids (haematoxylin lake after treatment by copper acetate) or for soluble soaps (fixation by formol + calcium salicylate to render the soaps insoluble; subsequent treatment by copper acetate and haematoxylin lake).

The fatty acids after having been rendered insoluble are also stained by anilin dyes, especially when these are in alcoholic solution and when they are heated. This is the case in Camus and Pagniez'[20] application of the Ziehl technique, in the Gram technique, or in the Altmann acid-fuchsin stain for mitochondria.

(2) Chromic acid and chromium salts. These act as strong oxidising agents upon fatty acids and lipoids, which are rendered insoluble, while they show no action upon soaps and neutral fats. According to Lorrain Smith, Mair, and Thorpe, this insolubilisation might be explained by the transformation of the fatty acids into more oxidised substances, less soluble in the fat solvents. Oleic acid, for example, would be changed into an insoluble dioxystearic acid. According to Lorrain Smith, Regaud and Policard, and Rubaschkin, a certain quantity of Cr remains fixed upon the oxidised products and acts as a "mordant" for a subsequent haematoxylin lake. Regaud's technique for mitochondria is based upon these principles of chromisation or post-chromisation. The same is true for the techniques of L. Smith, Dietrich, Ciaccio, and Bell. L. Smith and later Dietrich treated formol-fixed tissues with 5 per cent. potassium bichromate for 24–48 hours at 37° and stained with Kultschitzky's haematoxylin. He thought that in that way he was able to stain cholesterol-fatty acid mixtures, and cholesterol esters alone, but Kauffmann and Lehmann have recently demonstrated that lecithin must be present for a positive reaction to be obtained.

Ciaccio performs a chromisation at 37° for 6–8 days after fixation by particular fluids[1] (potassium bichromate, formol, formic acid, acetic acid) and stains with Sudan III at 37°. He thinks that he is able to detect lecithin alone, but Fauré-Fremiet, with the aid of his technique, shows the colorability of fatty acids too; Kawamura shows also the colorability of oleic acid (sodium oleate, cephalin, cholesterol and its esters being colourless). According to Kaiserling, however, these are stainable. Baginski[14] maintains that phosphatids in general are stained.

Bell stains tissues fixed in Tellyesnicki's fluid with Sudan III and thinks that the trioleins are in the form of ring-shaped droplets, while the lipoids appear as granules.

(3) The combination of both techniques (1) and (2) as in the Weigert technique (fixation by a chromic fluid and subsequent treatment with a fluid containing copper acetate; formation of a haematoxylin lake), or as in the method of Savini (5 per cent. copper bichromate at 37°; subsequent staining by Sudan III).

We can summarise as follows the reactions of the different fats, taking into consideration certain physical characters, such as solubility and refractive power (Table I).

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[1] I have remarked that this fixation is not very good. Dietrich's and Ciaccio's fixations may be replaced by Helly's fluid followed by a post-chromisation at 37° in saturated potassium bichromate. The results obtained are both histochemically and cytologically excellent (41).

Table I.

	Neutral fats	Fatty acids	Soaps	Mixtures of fatty acids and cholesterol esters	Mixtures of neutral fats and cholesterol esters	Phosphatids		Cere-brosid	Cholesterol esters and certain lecithins
						Cephalin	Sphyngo-myelin		
Sudan III	Red	Red	Red	Red	Red	Reddish yellow, very pale	Reddish yellow, pale	Reddish yellow, pale	Yellowish; disappearance of anisotropy
Scharlach	Red	Red	Red	Yellowish red	Yellowish red	Yellowish red, very pale	Yellowish red, pale	Yellowish red, pale	Yellowish or reddish, pale
OsO₄	Black indelible	Non-saturated: Black indelible — Saturated: } Grey brown.... (Staining intensity varying with the amount of oleic acid); lability. Secondary blackening by alcohol							
Neutral red	Negative	Red	Red	Negative	Negative	Red	Red	Red	Negative
Nile blue	Red	Blue	Blue	Lilac	Reddish blue	Blue	Bluish	Bluish	Reddish; anisotropy retained
Benda Fischler	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Fischler (Ca salicylate)	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative
Ciaccio	Generally negative	Sometimes positive	Negative	Negative	Positive	Positive	Negative	Negative	Sometimes positive
Weigert	Negative	Positive	Negative	Negative	Negative	Positive	—	Negative	Negative
Dietrich	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive

What have all these techniques demonstrated up to the present? Ciaccio[21] has well summarised our histophysiological knowledge. He classified the lipoids into three groups:

(1) Absorbed substances forming reserves utilised for combustion and perhaps for synthesis.

(2) Lipoids closely associated with the function of specialised endocrine glands (suprarenal cortex, corpus luteum, etc.).

(3) Fundamental constituents of cells, physiologically stable, varying in an appreciable degree only in abnormal conditions.

The first classes (1) and (2) are distinguished by their normally great variability, and they may be called in consequence "anabolic lipoids." The third class represents the lipoid tissue constituents or "histolipoids." These are phosphatids, cerebrosids and cholesterol. They are rarely free, being usually associated with proteins or with their decomposition products, which mask their true character and properties. Ciaccio proposes certain reactions (digestion by trypsin or treatment with phenol after fixation in formol; Cajal's and Da Fano's techniques followed by a chromisation) to produce an artificial "lipophanerosis," that is to say, to "unmask" the lipoids, thus bringing these substances into evidence. The idea is very interesting, and it is true that under certain conditions and in peculiar circumstances the existence of "unmasked" lipoids is very obvious. But we must remark that they were already visible with the ordinary techniques, as shown previously by Ciaccio[22] himself with his own first and classical technique, by L. Karpova[23], Parat and Painlevé[24], Parat and Gambier[25] on the idiosome of the male germ cells, by Regaud (1908–9), Fauré-Fremiet, Mayer, Schaeffer and Rathery, Fauré-Fremiet, Mayer and Schaeffer, Ciaccio, Mulon, Guilliermond, Parat and Bergeot[26], A. Giroud[8], etc., on mitochondria. The technique of Ciaccio produces an exaggeration of the phenomenon, but it is to be feared that the brutality of the technique (at least of that technique which is the best but also the most violent, namely digestion by phenol) leads the histochemist to pure artefacts.

As regards anabolic lipoids, I refer the reader to the papers of Mulon, Ciaccio[28], Baginski[14], Goormaghtigh[29, 30], Pighini[31], dealing with the suprarenal glands, the corpus luteum, the brain, etc. I must, however, draw attention to the "lipofuscines" of Borst, Hueck, or "chromolipoids" which were studied by Hueck, Mulon, and Ciaccio, who concluded that they are "oxylipoids," viz. auto-oxidable lipoidic substances which acquire a more or less deep colour in the course of this process of auto-oxidation. There is an excellent summary of these researches in the recent book by J. Verne[32].

B. SIMPLE ELEMENTS AND INORGANIC COMPOUNDS.

The histochemistry of certain simple elements and inorganic compounds is not sufficiently developed. Either the reactions are not suitable for tissues and cells or else the applications of the reactions have been too imperfect. This is frequently the case for sodium, magnesium, manganese, potassium, etc. For this latter element, however, chemical tests are good, as, for example, precipitation in the form of a

potassium cobalto-nitrite, or the action of double sodium and bismuth thiosulphate with subsequent elective staining by orcein (Cretin).

Among the best and most fruitful reactions, I shall mention only those for iron, phosphorus and calcium.

Iron. The reactions for iron are the best known and have been the most carefully analysed (MacCallum). It is not necessary to reconsider the ammonium sulphur (Quincke), Prussian blue (Perls) and Turnbull blue (Vogel) reactions. I must mention however an interesting contribution of Grynfeltt and Cristol (33). These authors have remarked, following the lead of Herxheimer, Schmorl, etc., that the techniques of Perls and Vogel were negative after fixations in fluids containing alkaline bichromates, as in Regaud's fluid. They neutralised the inhibiting action of the bichromate by precipitating it with a soluble lead salt (lead nitrate). This procedure permits of a very precise localisation of the iron in the cells, since a cytological fixation (Regaud, for example) may be employed.

Marcel Prenant (34) and Katsunuma (35) have demonstrated the presence of iron in intracellular granules which play a rôle in the Madelung (peroxidase) and Köchlin-Witt (oxidase) reactions, and which they suppose to be mitochondria.

A. Policard (36) has recently utilised "micro-incineration." He introduces sections cut by a freezing microtome and desiccated at a low temperature on a slide in a microcalciner. He demonstrates the presence of iron by the characteristic colour of the ashes (iron oxide); he obtains of course the *total quantity* of iron and is able by simple comparison with a control section to localise it, since it is incrusted *in situ* on the slide. Moreover this technique is appropriate for other elements, such, for example, as calcium (*vide infra*).

Phosphorus. The technique of MacCallum consists in treating the sections by the nitromolybdic reagent, and in reducing the phosphomolybdate thus formed by phenylhydrazin chloride. The blue colour obtained (due to molybdenum oxide) is, according to MacCallum, specific for phosphorus. Bensley, Arcangeli, Scott, Miller and Taylor have nevertheless criticised and condemned this method. Cretin (37) has proposed to apply the well-known precipitation of phosphorus by uranium (uranium acetate in acetified solution) to Histochemistry. Uranium being polyvalent is able to act as a mordant and to form a lake, for example with haematoxylin; this is the principle of the Cretin reaction.

A. Policard and Leulier (38) have also criticised these reactions as follows:

(*a*) The reagents are unable to unmask phosphorus in the tissues without destruction of all organic substances (Bensley, Scott, Posternak).

(*b*) By the action of the nitromolybdic reagent, the ammonium molybdate is adsorbed by the proteins and can be reduced to blue molybdenum oxide by phenylhydrazin chloride, even in absence of phosphomolybdate. The MacCallum reaction is therefore not specific.

(*c*) It is impossible to liberate and to characterise histochemically the infinitesimally small quantities of phosphorus included, for example, in nuclei or in cell granules.

Although this criticism is exaggerated on some points, we must confess that

none of the techniques for phosphorus detection is perfect. We must remark, however, that the MacCallum reaction gave positive results to Marcel Prenant (39), on Turbellarian rhabdites, and to M. Parat (40) on inclusions of foetal intestinal cells. These results were confirmed by chemical analyses.

Calcium. The classical reactions are:

(1) Oxalic acid: formation of calcium oxalate crystals. Accuracy: 0·06 milligram of Ca (Behrens and Kley).

(2) Sulphuric acid: formation of calcium sulphate crystals having a swallow-tail form. Accuracy: 0·04 milligram of Ca.

(3) Iodic acid (Denigès): formation of calcium iodate crystals.

But the topographical exactness is often far from perfect and it is sometimes difficult to identify the crystals. To these techniques we must add: the Kossa reaction for calcium phosphates with silver nitrate, the Stöltzner technique with silver nitrate and reduction by pyrogallol, the MacCallum or Koehl and Roth techniques based on the substitution of lead for calcium and reduction by sulphydrate of ammonium.

Recently, Cretin (42) has published a series of reactions which are summarised by Langeron (44). One of the best is the " gallo-formic " reaction to which Cretin (43) grants a sensibility of 0·001 per thousand.

A. Policard has applied his microincineration technique to investigations on calcium, especially in collaboration with Pillet (45). It is to be noted that the ashes obtained are not pure calcium; it is possible, however, to distinguish it, as done by Turchini (4), by the Denigès iodic acid reaction.

In any case, as Marcel Prenant (46) remarks, it is still difficult perfectly to localise the calcium in cells, in relation to the cell constituents. The microscopical anatomy of calcium distribution is well under way (Cretin, Policard, M. Prenant), but its cytological study is in its earliest infancy.

In conclusion to this review I must state that my intention has been not to give an exact list of histochemical techniques, but rather to point out certain of the methods and aims of Histochemistry, a science which is only in its infancy and whose evolution claims the attention of biologists.

BIBLIOGRAPHY.

(1) PRENANT, A. (1910). "Méthodes et résultats de la microchimie." *Journal de l'Anat. et de la Physiologie*, **46**, 343–404.

(2) —— (1921). "L'Histochimie." *Revue générale des Sciences*, **32**, 581–6.

(3) MANN, G. (1902). *Physiological Histology.* Clarendon Press, Oxford.

(4) TURCHINI, J. (1924). "Sur l'histologie et l'histophysiologie de l'oviducte de la poule." *C. R. Assoc. des Anatomistes, Congrès de Strasbourg.*

(5) DENIGÈS (1926). "Étude sur le réactif de Millon." *Bull. de la Soc. de Pharmacie de Bordeaux*, **64**.

(6) DERRIEN et TURCHINI (1924). "De la caractérisation cytochimique des enclaves albuminoïdes." *Bull. Soc. Sc. Médic. et biolog. de Montpellier et du Languedoc méditerranéen*, Séance du 15 Février.

(7) ROMIEU, M. (1925). "Sur la détection histochimique des substances protéiques." *Bull. d'Histol. appliquée*, **2**, 185–91.

(8) GIROUD, A. (1925). "Le chondriome; recherches sur sa constitution chimique et physique." *Arch. d'Anat. microsc.* **21**, 145–252.

(9) MEYER, A. (1920). *Morphologische und physiologische Analyse der Zelle der Pflanzen und Tiere.* Jena: G. Fischer.

(10) VERNE, J. (1921). "Les pigments tégumentaires des Crustacés Décapodes." *Thèse Sc. nat.* Paris, et 1923, *Arch. Morph. gén. et expér.*

(11) MILLOT, J. (1923). "Le pigment purique chez les Vertébrés inférieurs." *Thèse Médec.* Paris, et *Bull. biol. de la France et de la Belgique.*

(12) VERNE, J. (1923). "La réaction chromaffine en histologie, sa signification." *Bull. de la Soc. de Chimie biol.* **5**, 227–35.

(13) LEULIER, A. et NOËL, R. (1926). "Détection histochimique de la cholestérine." *Bull. d'Hist. appl.* **3**, 316–19.

(14) BAGINSKI (1926). "Influence de la résection du nerf vague sur la lipoidogénèse des caps. surrénales." *Bull. d'Hist. appl.* **3**, 185–98.

(15) FAURÉ-FREMIET, MAYER et SCHAEFFER (1910). "Sur la microchimie des corps gras." *Arch. d'Anat. microsc.* **12**, 19–102.

(16) FAURÉ-FREMIET (1911). "Sur la valeur des indications microchimiques fournies par quelques colorants vitaux." *Anat. Anzeiger*, **40**, 378.

(17) ZWEIBAUM (1923). "Sur l'utilisation du mélange 'nadi' et du bleu d'indophénol formé *in vitro*, en technique histologique...." *C. R. Soc. de Biol.* **89**, 256–8.

(18) ZWEIBAUM et MANGENOT (1923). "Application à l'étude histochimique des végétaux d'une méthode permettant la coloration vitale et post-vitale des graisses de la cellule végétale." *C. R. Soc. de Biol.* **89**, 540–2.

(19) MULON, P. (1904). "Action de l'acide osmique sur les graisses." *Bibliographie anatomique*, **13**, 208–13.

(20) CAMUS, J. et PAGNIEZ (1905). "Propriétés acido-résistantes des acides gras." *C. R. Soc. de Biol.* **49**, 701.

(21) CIACCIO, C. (1926). "I lipoidi considerati come constituenti essenziali della cellula. Nota prima—introduzione e tecnica. Nota II—distribuzione degli istolipoidi nei constituenti morfologici della cellula." *Bollettino della Società di Biologia sperimentale*, **1**, fasc. 1–2.

(22) —— (1910). "Contributo alla distribuzione ed alla fisio-patologia cellulare dei lipoidi." *Arch. f. Zellforsch.* **5**.

(23) KARPOVA, L. (1925). "Beobachtungen über den Apparat Golgi (Nebenkern) in den Samenzellen von Helix pomatia." *Zeitschr. f. Zellforsch. u. mikr. Anat.* **2**, 495–514.

(24) PARAT et PAINLEVÉ, J. (1926). "L'appareil de Golgi des cellules génitales mâles d'*Helix* et des autres Pulmonés." *C. R. Soc. de Biol.* **94**, 745–7.

(25) PARAT et GAMBIER, E. (1926). "L'appareil de Golgi des cellules génitales mâles du Discoglosse et du Cobaye." *C. R. Soc. de Biol.* **94**, 748–9.

(26) PARAT et BERGEOT, P. (1925). "Sur le prétendu contenu lipoïdique de l'appareil de Golgi." *C. R. Soc. de Biol.* **92**, 868–9.

(27) PARAT et HIBBARD, H. (1927). *C. R. Acad. des Sc.* (En préparation.)

(28) CIACCIO, C. (1915). "Untersuchungen über die Autooxydation der Lipoidstoffe." *Biochem. Zeitschr.*

(29) GOORMAGHTIGH, N. (1922). "Le cortex surrénal humain...." Doctorat spécial, Université de Gand, et *Arch. de Biologie.*

(30) —— (1926). "Étude histochimique du corps jaune de la chienne gravide." *C. R. Assoc. des Anatomistes, Congrès de Liège.*

(31) PIGHINI, G. (1915). *La Biochimica del Cervello.* Torino: Rosenberg et Sellier.

(32) VERNE, J. (1926). *Les Pigments dans l'organisme animal.* Paris: G. Doin.

(33) GRYNFELTT et CRISTOL (1923). "Procédé simple pour obtenir en cytochimie la réaction du Bleu de Prusse sur des organes fixés par les bichromates alcalins." *Bull. Soc. Chimie biol.* **5**, 797–800.

(34) PRENANT, M. (1924). "Études histologiques sur les peroxydases animales." *Arch. de Morphologie gén. et expér.*, Fasc. **21**.

(35) KATSUNUMA, S. (1924). *Intrazelluläre Oxydation und Indophenolblausynthese.* Jena: G. Fischer.

(36) POLICARD, A. (1923). "Sur une méthode de microincinération applicable aux recherches histochimiques." *Bull. Soc. Chim.* **33**, 1551–8.

(37) CRETIN, A. (1923). "De quelques méthodes de recherche du phosphore et de la chaux dans les tissus." *Thèse Médec.* Paris.

(38) POLICARD et LEULIER, A. (1925). "Étude critique sur les méthodes de caractérisation histochimique du phosphore." *Bull. d'Hist. appl.* **2**, 22–32.

(39) PRENANT, M. (1919). "Recherches sur les rhabdites des Turbellariés." *Arch. de Zoologie expér. et gén.* **58**, 219–50.

(40) PARAT, M. (1923). "Présence de Phosphore dans le méconium; son absorption par la muqueuse intestinale fœtale." *C. R. Soc. de Biol.* **88**, 606–7.

(41) ——,(1926). "Mise en évidence du chondriome et des lipoides cellulaires." *Bull. d'Hist. appl.* **3**, 222–3.

(42) CRETIN, A. (1925). *Recherches sur l'ossification et sur la réparation des os fracturés.* 351 pages, 13 planches. Imprimerie de l'Institut commercial, 4, Rue Auvray, Le Mans.

(43) —— (1924). "Sur un nouveau réactif du calcium applicable aux recherches histologiques." *Bull. d'Hist. appl.* **1**.

(44) LANGERON, M. (1925). *Précis de microscopie.* 4me édition. Masson.

(45) POLICARD et PILLET (1926). "Recherches sur le cartilage d'accroissement des os longs. I. Répartition histologique des matières minérales fixes étudiées par la microincinération." *Bull. d'Hist. appl.* **3**, 307–15.

(46) PRENANT, M. (1924). "Contributions à l'étude cytologique du calcaire." *Bull. biol. de la France et de la Belgique,* **58**, 331–78.

SEXUAL SELECTION AND ALLIED PROBLEMS IN THE INSECTS

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(With Seven Text-figures.)

CONTENTS.

1. ACKNOWLEDGMENTS AND INTRODUCTION.

In a group so large as the insects, it is always difficult to co-ordinate the numerous discoveries relating to anatomy and life history, while, on the other hand, the facts themselves become increasingly inaccessible if they cannot be arranged in some more or less logical system. Darwin's theory of Sexual Selection has been especially useful in this respect; for not only has it directed attention to

some of the most essential stages in all life histories, but it has systematised a part of the great mass of accumulated data. Since Darwin wrote there have naturally been large additions to this accumulation, but there have been few attempts either to incorporate them into his theory, or to test the old explanations with new examples. In insects, the best recent summary I know is Meisenheimer's (1921); the whole animal kingdom is reviewed, the insects being very fully treated, though of necessity many interesting questions are not considered in detail. The first part only of this work has hitherto appeared, so that there is little criticism of theory.

In the present paper, I have tried to bring together most of the essential facts and to criticise the explanations that have, at various times, been brought forward. In my opinion, ignorance on certain essential points allows no generalisation which includes all the facts. It is doubtful, indeed, if all secondary sexual characters ought to be treated together, since it is now quite evident that the sexes may come to differ from one another as the result of several distinct processes; these processes are discussed in the present paper, and some structures and types of behaviour are found to play a useful part in the life history, while others, whose significance is not known, can, at least, be put into several classes. Horn-like structures, for instance, on the head or thorax are, very probably, most often developed for the same reason in the various groups in which they are found; it is, at any rate, something to be able to class together all the examples of a given type of dimorphism, even if their cause is still mysterious.

To prevent misunderstanding, it must be explained that certain verbs, such as "choose" or "try," are used throughout to avoid clumsy circumlocutions. This seems to me all the more immaterial because the presence or absence of consciousness in insects does not affect the discussions of the present paper.

In the following account characters are called epigamic which are known by observation to be used in mating or its preliminaries. Some structures are, of course, epigamic without being sexually dimorphic. A complete treatment of the subject would deal with four types of structure and behaviour:

A. Sexually dimorphic: 1. Epigamic; 2. Not epigamic, but of known use (*e.g.* structures used by the female in ovipositing); 3. Of no known use.

B. Epigamic, but occurring in both sexes.

It would not be easy in all cases to apply this classification at all strictly, but it is useful in eliminating certain facts from the present discussion. Thus the second category need not be considered here, nor, in practice, need the fourth. Epigamic structures which are common to both sexes, generally have, in insects, other important functions besides their use in mating, so that their development would have proceeded without the influence of any kind of sexual selection. It is possible, however, that bright colours or conspicuous markings, found in both sexes, may sometimes have been acquired or retained mainly because of their importance in mating (see p. 313).

The line between Natural and Sexual Selection is not, of course, sharply marked, but some definition of their respective actions is necessary for the proper appreciation of the facts set forth in the succeeding paragraphs. A character that has been

acquired or preserved by the action of Sexual Selection must either be displayed to the other sex in courtship or used to drive away rivals. It has become obvious since Darwin wrote that display-characters are probably acquired most often as a result of Natural rather than Sexual Selection. The value of the display is to excite the female till she reaches a state in which she is ready to mate; when once this state is reached the female will mate with any male (see Sturtevant's observations on the Drosophilidae; the present paper, p. 311). The display is useful, therefore, not to the particular male but to the species, by reducing the time between the first meeting of the sexes and successful copulation. There is, as a matter of fact, little direct evidence that the display is really of survival value in this way, and that short delays would be very harmful, but it must be supposed that few insects can afford to waste much time when the weather conditions are propitious. Yerbury (1908) records that one of the pair of the fly *Poecilobothrus nobilitatus* F., whose courtship he was watching, was seized by a predaceous wasp before copulation had occurred. It would be interesting to obtain more evidence of this kind. In later paragraphs there is a discussion of why the female should be so unwilling as to need stimulation at all.

Structures that are definitely useful in fighting and are not used in dealing with the enemies of the species, may have been acquired as a result of true Sexual Selection. It will be shown (p. 304 *et seq.*) that in many cases males will attempt to displace their successful rivals when these are actually in copulation. This may have led to an increased efficiency in male clasping organs that were primarily developed through Natural Selection.

In many animals, and certainly most insects, there is a primary sexual dimorphism, viz. that the male is small and active, with a higher rate of metabolism, while the female is larger, more sluggish, and has a lower metabolic rate. These physiological differences are, of course, correlated with sexual dimorphism in the chromosome complex. In the case of insects, it has been shown conclusively that the gonads have no influence on secondary sexual characters or instincts. Thus a moth, which has had its own gonad removed and that of the opposite sex inserted, still develops into a normal specimen of the sex into which it was born (evidence summarised in Goldschmidt, 1923).

This dimorphism must lead automatically to other differences between the sexes which are of no direct use, *e.g.* in colour and shape. We find a good example of this in the Saturniid moth, *Callosamia promethea* Drury, examined by Mayer (1900). Here he found that the male moths would mate with females whose wings had been removed and had been replaced by those of males. The males took no notice of other males furnished with female wings, their reactions being entirely controlled by the scent given out by the female. Yet in this species there is a strongly marked colour-dimorphism, the male being black winged and the female red-brown; the Saturniidae is, further, a family in which the primary dimorphism in activity and size is well developed. Evidently these colour differences are secondary results of the primary dimorphism and are of no direct epigamic significance; the same must be true of many other dimorphic characters.

Thus in the humble-bees, the males nearly always have more yellow or white hairs than the female, especially on the head and thorax. This dimorphism is retained in all the polymorphic varieties in which each species is found. As far as is known, the males of these bees, when they are ready for mating, capture their females, so that there can be no question of the display of their somewhat brighter colours.

Emery (1884), further, in the glow-worm *Luciola italica* Scop., finds that a sexual difference in the colour of the prothorax is due to a difference in the colour of the underlying fat-body. Though the fat-body has its special colour in these forms as a consequence of the power of luminescence, the resulting colour of the thorax must be non-significant, since it cannot be seen in the dark.

There are a number of special cases of sexual colour dimorphism associated with mimicry, one of the most remarkable being that of *Papilio dardanus* Brown (illustrated in Eltringham, 1910). Here there is one type of male throughout the range of the species, while the females are totally different in colour and wing shape, and, in various geographical regions, resemble differently coloured models of other supposedly distasteful species. The coincidence in range between model and mimic has almost certainly been brought about by the action of Natural Selection, and the patterns of the mimetic females may also have been acquired in the same way. At any rate some factor, not Sexual Selection, is at work here, producing striking dimorphism.

2. MISCELLANEOUS, APPARENTLY USELESS CHARACTERS.

There are, however, a large number of structures amongst the insects, peculiar to the males, which seem too complex to be merely the secondary results of the primary dimorphism. Some of these, nevertheless, seem to be quite without use, and these will now be considered.

The mandibles of bees and wasps can be arranged according to structure and function into a very interesting series (cf. Darwin, 1894, p. 275). First in many species, *e.g.* the bees of the family Megachilinae, the male has smaller and weaker mandibles than the female, who has to excavate wood for her burrow. Secondly, the mandibles may be alike in the sexes (the sand-wasp *Ammophila*) or slightly more developed in the male than in the female (the sand-wasp *Cerceris*); here the females use their mandibles for excavation while the males use theirs for holding the neck of their partner when in copulation. Thirdly, the mandibles may be very strongly developed in the male, as in some of the bees of the genus *Andrena*. In certain species, such as those of the *Fulva*-group, the head is also much enlarged and the mandibles have a basiventral tooth, both these characters varying considerably within the species; here, too, the mandibles are used for holding the female. Finally in the wasp *Synagris cornuta* L. (see Bequaert, 1919, Plate 2), the mandibles, though larger in the male, vary much in size, the large males having them disproportionately increased in size. Very large mandibles, used for seizing the female, are also found in the male of the neuropterous insect, *Corydalis cornuta* L.; from the variation in the specimens I have seen, I suspect the variation here also

to be disproportionate. In such a series as the preceding it would be very hard to say where the action of Natural Selection ended and that of some other process, at present little understood, began. It would be difficult to show, and dangerous to assume, that in each case the degree of mandibular development was really correlated with needs of the species; for although some enlargement of the mandibles might be obviously advantageous, yet it is hard to see why they should be developed to the particular extent found in each species. In the case of *Synagris*, also, the great variability encountered makes it improbable that here the exact degree of mandibular development is important (but cf. Poulton, 1913 *b*).

It would be wearisome to detail all the strange structures found in male insects, but some of the secondary sexual characters raise great difficulties by their extravagance. The caddis-flies of the genus *Dipseudopsis* (Ulmer, 1925, p. 9, Fig. 12) have the spine at the apex of the hind tibia modified into unaccountable shapes. The fore tibia (*Rhynchotrichops aculeipes* Zett., Séguy, 1923, and *Campsicnemus magius* Lw., Lundbeck, 1912) of some flies has an extraordinary process of no apparent function. In the case of *Campsicnemus* (Lundbeck, 1912) the describer of the species was for some time ridiculed because it was suggested that he had mistaken a fungoid growth for a part of the fly.

Several of the large families of butterflies are distinguished from one another by a peculiar secondary sexual character. In the three families Lycaenidae, Erycinidae and Nymphalidae the front legs of the males become progressively reduced, the extent of this modification being indicated by the order of the names. In the Nymphalines the female as well as the male has this modification, though to a lesser extent. This character seems quite inexplicable, especially as it would be expected to make it less easy for the male to grip the female (Eltringham, 1910).

In two families of beetles the secondary sexual characters vary in an unusual way. In the Ipidae (Scolytidae), Hopkins (1894) shows that though the species are frequently sexually dimorphic, structures which in one species or genus characterise the male, may, in another genus or species, be peculiar to the female; it is only possible to sex these strongly dimorphic insects by examining the genitalia, since no analogies can be drawn from near allies. Secondly, in certain genera of the Bostrychidae, Lesne (1901) found that both sexes were usually recognisable by developments of the head, thorax and elytra; some of the males, however, varied in the direction of the female, acquiring many of her characters (as well as losing his own) and, in extreme cases, becoming scarcely distinguishable except by the genitalia. Sometimes there were two sharply marked classes of males, sometimes a graded series. It is difficult to believe that structures so variable can have any important direct significance to the insect.

3. HORNS.

Outgrowths of the head and thorax in the male are one of the best known types of sexual dimorphism, though examples outside the beetles are not very numerous. Smith (1922) figures the male of the lacewing, *Meleoma signoretti* Fitch, which has a relatively large horn above the antennae. Certain Ortalid flies, native to

New Guinea, have some of the most curious developments of this sort. In the genus *Phytalmia* (*Giraffomyia*) all the males have remarkable paired horns arising from the forehead; these horns are curiously like those of deer, being variously branched. In another genus, *Laglaisia*, the male has eyes at the end of stalks, which may be very long, but in some specimens scarcely serve to distinguish him from the female, whose eyes are quite normal. It is possible that in this case the length of the eye-stalk varies disproportionally with the size of the insect, but accurate measurement would be required to prove this.

In the preceding forms little or nothing is known of the biology of the insects, but it is probable that the horns are analogous to those of beetles, and probably, therefore, with no direct significance.

Amongst the beetles, the males of the Staphylinids *Bledius* (*taurus* Germ. etc.), *Platystethus cornutus* Gr., and *Siagonium* (*Prognatha*) *quadricorne* K. (see Fowler, 1888, pp. 362, 373, 433) have remarkably developed horns. These, in the first genus, are on the head and thorax, in the two latter, on the head alone, and here varying disproportionately with the size of the insect. The Silphid, *Agathidium rhinoceros* Shp., amongst other species of the genus (Fowler, 1889, p. 14) has the left mandible highly developed in the male and also very variable in size. The mandibles of certain male Prionidae are also enlarged and vary very greatly with the size of the animal (Gahan, 1889). The lamellae on the heads of some of the species of *Cis* and allied genera may also be mentioned (Fowler, 1890, p. 205); if these insects were not so small, the males would be very remarkable in such forms as *C. bilamellatus* Wood.

The extraordinary outgrowths on the head and thorax of many Scarabaeidae have never been satisfactorily explained. Bateson and Brindley (1892) record in *Xylotrupes gideon* L. that the large males have a cephalic and thoracic horn which can be used together like a pair of pincers; Baron von Huegel saw the large males seizing the females transversely in this way, and carrying them about with evident satisfaction. In this species, however, the small males have the horns scarcely developed, so that they are quite unable to hold the female like the large ones. The horns are also used in this way in *Chalcosoma atlas* L. and may be so in some other species, though more often there is not a pair of horns that can be apposed to one another. In any case it is unlikely that this function is very important, since the small males are quite numerous and have not disappeared as a result of selection. According to Arrow (1920) the species in which the two sexes combine to prepare the nest and food for the next generation have either a similar armature in the male and female or, at least, that of the male is not extravagantly different. This he tested by examining the front legs of numerous species; he found that the males provided with monstrous horns never showed any signs of wear in their tibiae as in the industrious species; they must be regarded as useless drones. Such forms are often much more brightly coloured than their respective females, and Arrow suggests that this, combined with their larger size and remarkable shapes, may attract the attention of enemies to them from the more valuable females. This explanation is not very satisfactory for selection against large, bright-coloured,

horned males would probably lead to a reduction of these characteristics in the male before the species as a whole would have had time to benefit it; it is better to suppose that there is one process at work throughout the Scarabaeidae tending to produce excrescences in the males, a process which is kept in check by selection when the male is required to do more than merely fertilise the female (cf. the Platypodidae, the present paper, p. 305). As in several of the previously described outgrowths, the horns of the Scarabaeidae are often much larger proportionately in the large males than in the small ones. Sometimes the shape of the horns varies very considerably with the size of the animal; when, in addition, this variation is bimodal, as in *Xylotrupes gideon* L. (Bateson and Brindley, 1892; they describe, also, the bimodal variation in the callipers of the male of the earwig, *Forficula auricularia* L.), the two forms appear at first sight like distinct species. This variability makes it unlikely that the horns can be of much direct use. The development of such structures is probably controlled by the existence of a logarithmic relation between the size of the outgrowth and that of the whole body (cf. Huxley, 1924). The occurrence of such a mechanism in a number of widely separated types of animals, and, in particular, the very specific shape of the fully developed outgrowths we are as yet quite unable to explain.

4. EVIDENCE FOR FIGHTS BETWEEN MALES.

As has been said on a previous page, we can most easily imagine sexual selection influencing structure as a result of fighting amongst the males; the evidence for such fights will be next considered.

Gruhl (1924) records many cases of male flies which are unable to recognise the species or sex of an insect flying by; the fly usually chases these suspected females, and may even attempt to copulate with males of his own or other species. Green (1921) and Morice (1921) record the same type of behaviour in bees. The frequency of such attempts must be remembered when so-called fights between males are described.

In various butterflies (*e.g. Limnas chrysippus* L., Marshall, 1902; *Planema alcinoe* Feld., Poulton, 1911) the males have either been observed to drive off other males from females which they were courting, or have attempted to replace a male already *in coitu*. Similar struggles can be observed in many moths, *e.g.* those which "assemble." The male of the purple emperor butterfly (*Apatura iris* L., Joy, 1902) has a favourite "throne" in the oakwood in which he lives, and other males are attacked on sight and driven away. In this case the fights take place in the absence of the female. Struggles of males for females are not rare in the Hymenoptera (*e.g. Bembix nubilipennis* Cress., Rau, 1918) where both sexes occur together in great numbers for a short season of the year and where the males are often much the more abundant sex in the early part of the day or at the beginning of the season. In the Chalcid *Nasonia brevicornis* Ashm., Alston (1920) describes the fights that take place when the males meet in their persistent searching for females. They use their antennae and fore legs, and frequently lose some of the

joints of the former. Sometimes the whole breeding cage seemed to be full of struggling males.

In the Central American Micropezid fly, *Cardicephala myrmex* Schin. (Wheeler, 1924 *b*), the males sometimes fight one another in the presence of females. The flies rise up on their long hind legs and push against one another with their "chests." Another Micropezid in Java (Wheeler, 1924 *b*) fights in similar way.

Gruhl (1924) records a few cases of fights amongst male Empidids, *e.g. Empis opaca* Mg. In these flies the males fight, not for the females, but for the prey which each male has to present to the female at the moment of mating. In the case of the fly mentioned, one of the rivals was killed.

The New Zealand mosquito *Opifex fuscus* Hutton (Kirk, 1923) has peculiar mating habits which lead to fights amongst the males. The males hatch out first and seek for the floating pupae of the females; when a mature pupa is found it is slit open with the genital forceps and the female is fertilised before she has quite left her puparium. When a male has taken possession of a pupa he may have to defend it against other males, using his proboscis. Finally the males of the Drosophilid fly *Zygotricha dispar* Wied. (Bristowe, 1924) have a curiously developed head, which is much produced laterally, the eyes themselves forming short horns. These males fight for the females, butting into one another with their heads.

Darwin (1894, p. 299) mentions some instances of combats amongst beetles. In particular he gives some evidence that the males of the stag beetle (*Lucanus cervus* L.) fight in the presence of the females and also when alone. It remains, however, doubtful how far such fights take place in nature, and especially whether the fights are not due to general pugnacity rather than to a desire to possess a particular female. My friend Mr A. H. Hamm, who formerly lived in a part of Berkshire where the beetle was very common, tells me he has never witnessed a combat; it must be admitted, however, that these beetles are chiefly active at night. No doubt, also, the same process which leads to heterogonic growth in the Scarabaeidae is at work here, larger males having disproportionately larger mandibles (cf. also Dudich, 1923, *Cyclommatus tarandus*, Thb.). Poulton (1913 *a*) records an observation by Lamborn of the copulation of the Lycid beetle *Metriorrhynchus semiflabellatus* Thoms., when three unsuccessful males were struggling to oust the one who had actually gained the female.

Hubbard (1896) records severe fights between males of the social beetles of the genus *Platypus* (Platypodidae). In these the elytra have spine-like projections behind in the male. Hubbard says (*loc. cit.* p. 425) "the female is frequently accompanied by several males, and as they are savage fighters fierce sexual contests take place, as a result of which the galleries are often strewn with the fragments of the vanquished. The projecting spines at the end of the wing-cases are very effective weapons in these fights. With their aid a beetle attacked in the rear can make a good defence and frequently by a lucky stroke is able to dislocate the outstretched neck of his enemy." In this case weapons would appear to have been acquired through Sexual Selection. In the Platypodidae, however, the problem is complicated by the effects of the social life of some of the species. If the male helps the female in

looking after the larvae, as in *Xyloterus* (Hubbard, 1897), the sexual dimorphism is slight or absent; marked dimorphism only occurs in the species in which the males are useless drones. In other species (Strohmeyer, 1911), the females would appear to have been modified, being provided with structures for carrying the fungus on which the larvae and adults feed, to new nests.

Darwin (1894, p. 289) has already described fighting amongst crickets; this is confirmed by Fabre (1899), who adds that the fights are definitely for the possession of the females. Poulton (1896) has described a combat between the males of the grasshopper, *Arcyptera (Stethophyma) fusca* Pall. The Orthoptera, however, do not often appear to have any special weapons for their attacks. Hudson (1920) records that a species of *Deinacrida*, which is polygamous, has a very large head and mandibles in the male; fights have not yet been witnessed.

In most of the preceding cases there are no special structures developed for fighting, nor, in many of them, is there any marked dimorphism of any sort. If, in any insect, for one reason or another, there are many males and few females in a given locality, then there are sure to be some struggles between the males. There is, however, little evidence that special structures have often been acquired as a result of these fights; sometimes, where structures are found which look as if they were meant for fighting (*e.g.* in the Scarabaeidae), no fighting has been recorded.

5. APPARATUS FOR GRASPING THE FEMALE.

The common type of struggle, in which males try to dislodge a male already copulating, might be expected to lead to very efficient clasping organs in the male. Some of the latter will now be described.

Many male insects have the front legs more or less expanded into a disc for gripping the female; this is well known, for instance, in the water-beetles (*Dytiscus*, etc.), in various solitary wasps (Crabronidae), or bees (*Megachile*), and grass-hoppers (*Gomphocerus sibiricus* L.). In other forms, some of the other legs have structures developed for the same purpose. Thus in the wasp *Hoplomerus spinipes* L., the mid-femora have certain spines which, as Chapman (1870) has shown, fit in between the nervures of the female's wings, so as to grip them tightly during copula-tion. In other cases, as in the beetle *Osphya* (Edwards, 1907), the male hind femora are modified to grip the elytra and abdomen of the female. Again, the antennae may be distorted at a certain point, so that the male may grip those of the female in the crook so formed (*e.g. Melittobia*, Hymenoptera, Balfour-Browne, 1922; *Meloe*, Coleoptera, Fowler, 1891, p. 93; *Sminthurides*, Collembola, Handschin, 1926). In some of the Collembola, also (Handschin, 1926), curious processes on the anal segment of the female seem to be used to help the male to retain his position. It was formerly thought that the furrows or special sculpture found on the elytra, of the females only, of water-beetles (Dytiscidae)[1] were for the same purpose; this is now known not to be the case, the modified part of the female not being gripped by the male at all. The females, further, of several species are strongly dimorphic, some

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[1] The following papers deal with the sculpture of the Dytiscidae: 38, 347, 350. The numbers in this and other footnotes refer to the numbers given to the papers in the bibliography, pp. 350-60.

being like the male, and at present the secondary sexual sculpture of water-beetles seems to be a striking example of an apparently useless character.

In a somewhat special category are the prehensile organs developed at the end of the abdomen in nearly all male insects. From their structure it is obvious that these must be very efficient claspers, but there is considerable difficulty in explaining their extreme diversity, and especially their highly specific character. There is very little evidence that in each species they are so constructed as best to fit into the female; in *Bombus*, for instance, Boulangé (1924) points out that the most complicated part of the apparatus does not enter the body of the female but clasps a part of her which is alike in all the species. There is a similar difficulty in explaining the specific shapes of the expanded tarsi of such forms as the species of the Crabronidae, and also, in this case, the irregular way in which such tarsi are found in one species and not in the next. At present we must leave these problems unexplained.

E. M. Walker (1912 and 1915) has shown that in certain dragonflies (*Aeshna* spp. and *Staurophlebia*) there is sometimes special adaptation of the male anal appendages to the needs of mating. In the dragonflies, as is well known (see photograph; Holland, 1922), the male grips the head, and sometimes also the prothorax, of the female, who is fertilised by bending her abdomen up to the males second ventral segment, where there is a secondarily developed copulatory apparatus, in which he has already deposited his sperm. In the forms mentioned above, the nuptial flight is particularly wild and erratic, and the male appendages have a shape that enables them to hold the female with greater firmness than is usual. Walker does not say whether there has been any co-adaptation in the females; presumably not, since (1912) he records the coupling of distinct species in two cases. This special male adaptation is of generic or sub-generic and not of specific value.

The organs for clasping the female would in any case have been developed because essential for the reproduction of the majority of species, but it is possible that, in some cases, the necessity of hanging on in spite of the attempts at dislodgment made by other males may have led to the claspers, etc., being more efficient than they would otherwise have been. If this is the case, then a kind of Sexual Selection has occurred. It will be pointed out in a later paragraph that the struggles to dislodge successful males are very likely the result of some special scent given off during copulation which is more attractive to other males than that of an unmated female.

6. DISPLAYS BEFORE THE FEMALE.

(a) *General.*

Displays can be divided into four types according as the appeal is made through the sense of sight, touch, hearing or smell (or taste). In many cases the behaviour of an insect combines several of these types of display, so to avoid repetition the classification will not be strictly applied.

Amongst the flies, the males of certain species of *Thereva* (*annulata* F., Therevidae), and of *Argyra* (Dolichopodidae) have the thorax and abdomen dusted with white, appearing silvery in the sunshine. In the first-named species Verrall (1909)

records that "males execute a wild frantic dance in groups numbering up to eight or ten individuals over the hot sand in bright sunshine at from four to six feet from the ground." Adlerz (1912) also mentions that the males of a *Thereva* (unidentified, but either the preceding species or *Th. lunulata* Zett.), in Sweden, fly up into the air on sunny days and fall down like snowflakes. Probably in both these cases the females are in the neighbourhood, though this is not stated. At any rate in *Argyra*, where the silvery males perform similar wild dances, the females sit about on the herbage near by (Lundbeck, 1912, p. 332).

Some of the most elaborate courtship ceremonies are those described by Gruhl (1924) amongst the Dolichopodids[1]. In some respects the behaviour is strongly reminiscent of that of courting spiders. The displays are divided by Gruhl into two types, one being made on the wing, the other when standing near the female. I think it would be convenient to consider, also, as a separate type, the display of specially modified parts of the legs. There is only space here to give a brief outline of some of his observations.

One species, almost certainly *Poecilobothrus nobilitatus* F., was observed displaying on the surface of the water in a ditch, in northern France. In his "pedestrian" display, the male stands in front or to one side of a female and spreads his wings far forwards, holding them for a short time in this position, and vibrating them three or four times, a movement made conspicuous by the milk-white spots at their apex. This performance is repeated several times with repeated changes of his position with reference to the female. His motion gradually becomes quicker, and, rather suddenly, he begins his aerial display. In this he hovers for a moment on one side of the female, with his wings beating rapidly, then dashes to her other side, where he hovers again. These two types of display may follow one another several times and it is uncertain whether they indicate different degrees of excitement in the male. The females appear quite inattentive to the males, and successful pairing was only seen twice; the male dropped on the female when he was hovering and copulation lasted at the most only five seconds. Gruhl describes very similar behaviour in a dozen or more other species.

Another Dolichopodid, *Neurigona quadrifasciata* F., was found abundantly on the trunks of alders in Germany. The females sat motionless on the trunks, while the males maintained a restless search for their mates. The females could be divided into two types, according to the way in which they sat; in one the abdomen was long and straight, while in the other the abdomen was much thicker and was bent. It was noticeable that the males paid most attention to the second type of female. If such a one was found, the male would stand behind her, raising himself on his long hind and mid legs. The front legs were extended straight forwards, so that the expanded basal joints of the front tarsi were held one on each side of the female's eyes. The front tarsi were then waved up and down, and at each movement the wings were spread forwards and vibrated, then returned. This was repeated 20–30 times, the female apparently taking no notice, and the performance ended in a sudden beating

[1] The following papers describe the courtship of the Dolichopodidae: 4, 57, 75, 89, 155, 234, 405.

of the wings, during which the abdomen, which had hitherto been directed obliquely upwards, was bent forwards under the thorax, and the front tarsi were held motionless in front of the eyes of the female. These tarsi were then quickly placed at the base of the female's wings, and the body was bent back so that the front legs were taut, the abdomen being stretched still further forwards to allow the genitalia to come into contact with the end of the female's abdomen. Copulation now took place, if the courtship had been successful.

The display of several other species is described, in which peculiar, secondary sexual modifications of the male's legs are used in an elaborate display. Doane (1907) in *Scellus virago* Ald., a fly of the same family, found that the female played the active part in the courtship, the male eluding her, just like a coy female. In other respects, however, this fly did not have a very different courtship from many other species of the family.

Aldrich (1906) has figured the hind legs of the species of the genus *Calotarsa* (Platypezidae); the tarsi are adorned with clubbed hairs and some of the joints have strange processes, the whole forming a structure very distinctive of each species, and remarkable even for the secondary sexual character of an insect. It is probable that these are exhibited to the female in courtship.

Wheeler (1924 *b*) has given an account of the curious dances of the males of the Micropezid, *Cardicephala myrmex* Schin. If a male finds a willing female, he approaches, facing her, to within a few centimetres; then he steps first to one side then to the other, swaying his abdomen towards her, and tapping with it on the leaf, till finally he jumps on her back.   .

Since the paper by Aldrich and Turley (1899), it has been known that the Empididae[1] practise some of the most complex mating ceremonies of all flies, perhaps of all insects. Certain Empidids, such as those belonging to the sub-genus *Xanthempis* Bezzi (Hamm, 1909 *b*), are ordinary predaceous insects in their behaviour. When the time for mating arrives, for instance, in *E. trigramma* L., the male seeks out the female and alights near her. He flutters his wings for a few seconds, and then raises his anterior legs and waves them about in front of him. The female replies with similar movements. The male now rubs his front tarsi together at a considerable rate, at the same time rapidly vibrating his wings which are held horizontally. This is repeated by one or the other sex for about three minutes. The pair have now drawn closer together and are able to touch one another's tarsi in a caressing manner. This they do continuously for about two minutes, the male every now and then rapidly vibrating his wings. Finally the female slightly raises the end of her abdomen and the male flies gently on to her back and copulation ensues. The only special point in this courtship is that the female is nearly as active as the male.

In most of the other species, however, belonging to the sub-family *Empidinae* there is a much more elaborate courtship in which the male catches some insect,

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[1] The following papers deal with the secondary sexual characters, the prey and the courtship of the Empididae: 5, 155, 159, 160, 161, 187, 233, 235, 249, 258, 301, 306, 392, 398. Papers 155 and 398 may be consulted for the early literature.

not as food for himself, but to hand over to the female at the moment of copulation. This behaviour is now well substantiated (the evidence is given in a collected form by Gruhl, 1924). In the more specialised forms, *i.e.* species of *Hilara*, the prey becomes gradually less important, its place being taken by a web or mass of loose threads spun by the male. In some forms the prey is embedded in the web but is often lost or is replaced by petals of flowers, etc., picked off the surface of the stream over which the male has been flying (*H. maura* F.), while in other species (*H. sartor* Beck.) the web is of a much more compact texture and is never provided with prey. In *Empis aerobatica* Mel. (*poplitaea*) (Aldrich and Turley, 1899) the male makes a "balloon" of bubbles in which a small fly is usually embedded.

The transference of the prey is usually preceded by an aerial dance, in which the males alone participate (*E. opaca* Mg.), or both sexes (*Hilara* spp.), or the females only (*E. livida* L.). As has already been noted, the females are more active than is usual in insects; it is therefore interesting to find that, in some species of *Empis* and *Rhamphomyia*, the females have their legs provided with broad scale-like hairs, arranged in regular dense rows, which, to human eyes at least, have a very decorative effect. In the female, further, of the New Zealand species of *Hilara*, *H. flavinceris* Miller, the membrane of the third abdominal segment is produced into an extensible bladder, standing out on each side (Miller, 1923). The males, on the other hand, seem, on the whole, to be without striking secondary sexual characters in this family. This brief account illustrates the remarkable courtship of the Empidids, part of which is certainly a "display," *e.g. E. trigramma* L., or the dances performed by one sex only. The full meaning of this behaviour is most easily understood when considered in conjunction with that of various other insects (see pp. 328 and 343).

Sturtevant's observations on the Drosophilidae (1915 and 1921) are particularly valuable because they show the amount of differentiation within the family and because he showed by experiment the true meaning of the display. The courtship of *D. melanogaster* Mg. is one of the most complex. Here the first sign of sexual excitement in the male is the extension of one wing at right angles to his body and the rapid vibration of it for a few seconds. This "vibration" is repeated at intervals throughout courtship until copulation occurs, and is done now with one wing, now with the other. Between the vibrations there is a slow, partial spreading and closing of the wings, termed "scissor movement." During both these movements the male faces the female, but he may face any part of her. Usually he walks round her in a semicircle several times, facing her as he moves, a process called "circling." He now licks the ovipositor of the female, and next bends his abdomen in such a way as to bring his genitalia under his thorax and jerks them towards those of the female. If he is successful in copulating, he then mounts on her back, between her wings, and holds on to her thorax, wings or abdomen with his legs.

Sturtevant found that amongst other allied species these movements might be permuted in various ways; some of them might be omitted or the female might make answering signals. In the genus *Chymomyza* there was no courtship, but the male rushed at the female and seized her wings between his front femora and

tibiae, the former of which are provided with a comb of bristles, probably for this purpose.

Sturtevant (1915) also made some experiments with *D. melanogaster* Mg. It was found that a male whose wings had been cut off, if confined with a female, would mate successfully though he took longer than usual to persuade the female to allow him to copulate. If a wingless and a winged male were introduced together, the duration of the courtship was normal and the female would mate almost as often with one male as with the other. The only possible interpretation of this experiment is that the function of the wing movements is to excite the female, and that once the female is sufficiently excited she will accept any male.

Pérez (1911) in *Chloria (Chrysomyia) demandata* F., a fly of a quite different family—the Ortalidae, records a very similar courtship. This consists of "circling," tapping the thorax of the female, licking her ovipositor, and then copulation tail to tail. Lindner (quoted by Gruhl, 1924) also observed this species, but he gives a rather different account. After the tapping of the thorax, he says, the male vibrates his wings and then quickly jumps on the back of the female for copulation. Possibly, as Gruhl (1924) suggests, two different species were observed by Pérez and Lindner respectively.

Tillyard (1917, p. 325) describes a display in certain dragonflies. In the first, *Rhinocypha fenestrella* Ramb., the mature male has the surface of his tibiae whitened and he displays them while dancing before the female. The male of *Calopteryx maculata* Beauv. possesses a shining white ventral spot at the tip of his abdomen which he displays by curving the abdomen upward and forward, the fore wings being held motionless and the hind wings fluttering rapidly. A more prolonged courtship is found in the metallic dragonfly *Hemiphlebia mirabilis* Selys. This species is almost invisible when seated on reed-stems except for its long, ribbon-like, white, anal appendages. These are displayed to the female by raising the abdomen and bending it slightly sideways while walking up a reed. The female replies by moving her abdomen from side to side in a peculiar manner. Finally they fly out from the reeds and engage in a *pas de deux* before pairing.

Mayflies have long been known to have a well-developed display flight. The males gather in swarms, in the genus *Ephemera* for instance, and rise and fall in the air through a distance of about 6–20 feet. The females, which often seem to be locally or temporarily rarer than the males, fly through such dancing swarms horizontally and are at once seized by a male. These swarms are very conspicuous and probably act as recognition marks for the species. In one species, *E. danica* Müll., the abdomen is milk-white in the male, and less distinctly white in the female. On the whole, however, sexual dimorphism is not very marked in these insects except in the front legs, which in the male are specially developed for grasping the female, and the eyes, which he needs for seeing her (cf. p. 340).

Even in the Collembola there may be some sort of display. Darwin (1894, p. 279) quotes the well-known passage from Lubbock (1871), describing the habits of *Bourletiella (Sminthurus) lutea* Tullb. "It is very amusing to see these creatures coquetting together. The male, which is much smaller than the female, runs round

her, and they butt one another, standing face to face, and moving backwards and
forwards like two playful lambs. Then the female pretends to run away, and the male
runs after her with a queer appearance of anger: gets in front and stands facing her
again: then she turns coyly round, but her quicker and more active mate scuttles
round too, and seems to whip her with his antennae: then for a bit they stand face
to face, play with their antennae and seem to be all in all to one another." In
some of the other species described by Handschin (1926, p. 34) there are fewer
preliminaries, but the male has a special apparatus for gripping the female.

(b) *Luminescence.*

A very specialised type of display is seen in the luminous beetles of the families
Lampyridae and Elateridae[1]. The types of organs met with in these families and
their employment in bringing the sexes together have recently been discussed by
Blair (1924 and 1926). When present at all, the faculty is usually found in both sexes,
the organ being situated, in the Lampyridae, on the last two abdominal sternites,
in the Elateridae, at the hinder angles of the pronotum and at the base of the
abdomen. In the genus *Phengodes*, the railway beetles, now separated as a distinct
family from the Lampyridae, some of the females and larvae have a red light on the
head and rows of green lights along the body. In the Lampyrids according to
Gorham (1880) the species can be divided into three types according to their sense
organs. In the first the antennae are plumose, the eyes moderate or small, the
luminescent power is small, and both sexes are winged. In the second, the antennae
are usually filiform, the eyes are large, sometimes excessive, the light emitted is
considerable, sometimes greater in the female, and again both sexes are winged.
Finally in the third type the antennae are usually rudimentary, the eyes are large, in
the male often excessively so, and the light is often very great in the female, in
which sex also the wings are reduced or absent. This arrangement of the species
seems to show the transition from insects in which the sexes are brought together
mainly by a chemical sense as in the related Drilidae (cf. p. 323) to those in which
luminescence is the most important agent.

Observations quoted by Blair make it obvious that the sexes really are brought
together by their perception of the light emitted. Thus MacDermott and others have
shown in America, that specific differences occur in the colour of the light or, in
some cases, in the periodicity of the flashes, and that, where two or more species
occur together, a given female will usually only reply to the flashes of the appro-
priate male, while a flying male will only respond to the light of his own female.

Observations of Withycombe on *Pyrophorus*, an Elaterid (Blair, 1926), show that
here, too, the males find the opposite sex chiefly by the light the latter are able
to emit.

In those species in which the light is given out in flashes, and is entirely under
the control of the insect, as in *Photinus*, the function of the light is particularly
evident. It is, in the male, a stimulus to induce the female to make the signal by

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[1] The following papers describe the phenomena of luminescence: 11, 34, 35, 36, 48, 67, 121, 139,
150, 152, 177, 211, 236, 237, 238, 239, 240, 283.

which he finds her. Darwin (1894, p. 277) considered that the power of luminescence was a warning signal, especially as it is in some species possessed by the larvae as well as by the adult; Belt, he says, has shown that the Lampyridae are distasteful to birds. I am inclined to think that the larval luminescence is in origin, perhaps, only an accidental result of the developmental process which leads to the adult structure; but however this may be, there is no doubt as to its main function in the perfect insect of most species. Paiva (1919) shows that the larva of an Indian *Lamprophorus* glows in the daytime only when noises are made near it, so there may be a real warning function in some species.

A short discussion of the synchronous flashing of fireflies will be found on p. 318.

7. DIMORPHISM IN EYES.

It is convenient, when considering displays, to describe the sexual dimorphism found in the eyes of insects. A great number of flies have eyes bigger in the male than in the female, so that in the former sex they may meet on the top of the head; in some cases, as for instance in *Therioplectes distinguendus* Verr., the eye is also divided into two sharply defined areas, in the lower of which the facets are of smaller size. In mayflies, also, a similar specialisation of the eye occurs in many males, and, in the family Baetidae, the eyes are often completely divided into two, one half being directed upwards, the other laterally and downwards. In this case the structure is probably correlated with the manner in which the male seizes the ventral surface of the female when pairing, and so has to be able to see her when he is flying below her.

In other groups dimorphism of the eyes is much more sporadic. It has already been mentioned in the luminescent beetles. It is found, too, in the sand-wasp *Astata* and also in certain humble-bees (*B. mendax* Gerst. and *confusus* Schenck).

Probably most cases of this type of dimorphism are correlated with some kind of "marriage by capture" in the male. This certainly seems to be the case in the flies, for those species with the most elaborate courtship seem to lack the enlarged and approximated eyes. In the case of the two genera of hymenoptera, the male insect has a peculiar, Syrphid-like, flight, hovering motionless, and darting after the females (see Saunders, 1909).

8. USE OF COLOUR IN DISPLAYS AND IN SPECIES RECOGNITION.

In many insects the wings are fluttered before and during mating, and it might have been supposed that, as in some Dolichopodids, the wings of the male would often have marks that would be exhibited by these movements. This, however, does not seem to be the case. Even in those forms (*e.g.* in the many Drosophilid flies examined by Sturtevant, 1921) in which the male has specialised wing movements which he performs before the female, there is often, probably usually, no dimorphism in wing colour. Conversely in species with distinctive sexual marks in the male (*e.g.* the orange tip butterfly, *Anthocharis cardamines* L.) there is often no record of a special exhibition before the female.

It is likely that in many bright coloured insects, the colours, though not acting as a specialised sexual stimulus, may yet be very useful as recognition marks. Thus Eltringham (1919 *a*) found that the males of the butterfly *Brenthis euphrosyne* L., in which both sexes are alike, would examine attentively coloured cardboard models of the species that had been placed on the ground in a wood where the butterfly was abundant in the mating season. They were also observed to take some interest in a bud-scale of approximately the right colour; each passing male would dip down to take a nearer view. Seitz (1913) also carried out rather more elaborate experiments along the same lines; he was able to show, in the butterfly *Anthocharis charlonia* Dup., that the males could distinguish models that differed in either colour or size from those that had a close resemblance to the female; with the accurate models the males would make prolonged attempts to copulate. Probably the use of colour for species recognition is frequent in butterflies; thus when males chase specimens of other species it is usually those which resemble them in colour.

Amongst the Hymenoptera, I have noticed (near Oxford) that the males of the sawfly *Allantus temula* Scop. pounce from above on specimens of either sex of the species when seated on flowers. They take no notice of other, differently coloured, species of the genus. If a male pounces on another male there is a short struggle and they separate; if on a female they usually copulate. There is considerable dimorphism in colour, the male venter being mainly yellow while that of the female is mainly black, but this difference seems to be of no significance in courtship.

It may be added here that, even when both sexes are alike in colour, the colours may originally have been modified for stimulating the female. If bright colours do in fact stimulate the female, then they might be acquired primarily by the male and the usual type of hereditary mechanism would, in many cases, ensure that the character was "transferred" to the female. Thus in many flies of the family Sepsidae, and *Seoptera vibrans* K. (Ortalidae), in the former of which, at least, there are conspicuous wing movements before mating, the wings have a well-marked black spot near the apex. On the other hand, in the brightly coloured butterfly *Vanessa urticae* L., Poulton (1904 *a*) shows that the most important part of the display by the male consists in tapping the hind wings of the female with his head, colour probably being unimportant as a sexual stimulus. (Cf. also, Cochrane, 1909.)

It is probably true to say that marked colour-polymorphism (whether restricted to one sex or not) can rarely occur in an insect in which the colour differences are the basis of a display by the male and in which also sound or scents do not play the principal part in mating. At any rate those polymorphic forms which have been examined from both points of view (*e.g.* many grasshoppers; some Syrphid flies; some Nymphaline butterflies) always make special sounds or scents the basis of their courtship.

9. USE OF SOUNDS IN DISPLAYS.

The power of producing sounds is found in many groups of insects, frequently being common to both sexes, as in the wasp *Mutilla*, or as in many beetles. In

most grasshoppers and crickets, and in the cicadas, however, this power is re-stricted to the males, and, in the first two, a complex auditory apparatus is also developed. Apparently in the Ephippigers the sound-producing mechanism is equally efficient in both sexes (Petrunkewitsch and Guaita, 1901) or even rather better in the female. These authors also state that the females of many species have a rudimentary, though possibly functional, sound-apparatus of a different nature from that of the male; I cannot, however, find any observation of the use of this organ in the live animal. In the males, the sound is produced in different ways in each group, and the methods are much modified in particular genera; structures for increasing resonance have also been developed independently on several occasions, *e.g.* in the cicadas, the Ephippigers, and the grasshopper *Pneumora* mentioned by Darwin (1894, p. 287). In some grasshoppers (*e.g. Psophos stridulus* L., *Stauroderus scalaris* Fisch.) there is an additional mechanism by which the wings make a loud rattle when the male, who, in these species, is more aerial than usual, is travelling through the air.

There is ample evidence that the males stridulate to excite the female and also, sometimes, in rivalry with one another. There is often a noticeable difference between genera and species both in the intervals at which sounds are uttered, and in the particular note. In some cases (*Psophos stridulus* L., Poulton, 1896) the male has a special call which he utters only in the presence of the female. Poulton's observations also show, in several other species of grasshopper, that the male only stridulates at all, or, at least, persistently, in the presence of the female, and that sometimes one or more males will stridulate near the same female. I have similarly observed competitive stridulation between two males of the grasshopper *Stenobo-thrus lineatus* Pz., one being seated on each side of the female, about half an inch away from her. Darwin (1894, p. 283) records that male crickets call the females from their burrows by their song, and also (p. 282) describes the rivalry between the males of cicadas. Poulton (1921 *b*) also records that males of the cicada *Mono-metopa insignis* Dist. call in rivalry in close proximity to the females. In this species, also, there was evidence that the sound had to be made for some time before the female became acquiescent. On one occasion a male attempted to copulate too soon, and was unsuccessful; he moved back to his original position, and continued singing to the female, who remained by him. Hudson (1920) also notes the rivalry of the males in the New Zealand cicadas.

It is difficult to say whether the rivalry between males in these forms has really led to any structural modifications. The stridulation is evidently useful in bringing the sexes together and in stimulating the female and so shortening the period during which the male has to wait before he is allowed to copulate; on the whole the existing evidence would not seem to show that rivalry was very important.

In the stoneflies (Perlidae) the males of certain genera have a callosity on the ninth sternite with which they beat the surface on which they are seated so rapidly as to produce a sound audible at 15–20 feet (Macnamara, 1926). This noise is only made in the dark and probably results in the sexes finding one another; the males "drum" in the absence of the females. Somewhat similar to this drumming is the

tapping of the death-watch beetle, *Xestobium rufovillosum* De G. (*tesselatum*). This is well described by Darwin (1894, p. 306), who says that both sexes make the noise (banging their heads against the substratum), and that it leads to pairs finding one another and copulating. A comparable example is the Psocid, *Atropos pulsatoria* L., long known as the lesser death-watch, whose habits have at last been described in detail by Solowiow (1924). These insects knock their bellies on the substratum, producing a noise which is quite audible, especially if they are seated on loose wall-paper, which acts as a resonator. They are able, also, to make a different sound by giving a series of taps in more rapid succession.

In flies there is no special male apparatus for sound-production, but the male is able, frequently, to raise the note of his humming to a higher key, characteristic of courtship. This is the rule in Syrphidae, and is also recorded in the Bombyliidae (Poulton, 1918 *b*), and Asilidae (Poulton, 1918 *b*; Melin, 1923).

In the Asilids (*Promachus* sp. Poulton, *Dioctria atricapilla* Mg., personal observation; also Melin, 1923) the male hovers in front of the female, swaying slightly, either up and down or from side to side, humming on a very high note, which gradually becomes louder and louder; the female appears to watch him intently, and, when the affair seems about to reach a climax, flies off with the male after her, and the performance is repeated. The actual mating has not been witnessed. In the Bombyliid *Exoprosopa eluta* Lw. a very similar display was witnessed, though here the humming is described as having a rattling element in it, like an aeroplane heard when high up.

In the Syrphid *Merodon equestris* F. I have seen the two sexes hovering in the air opposite to one another, as if they were each suspended from a string; at regular intervals they knocked their heads together and then swung apart again. As they did this they slowly sunk to the ground, the male maintaining the whole time a shrill hum, as he does, also, whenever he thinks he sees a female. In one case the male was seen attempting to mate with a worker honey-bee. The bee was heard by the fly when it was gathering pollen on a cornflower; the fly immediately started hovering on a level with the bee, about two inches away from it, at the same time raising the key of its humming. Finally the fly dashed at the bee and knocked it off the flower; the bee was twice knocked down before it was able to escape. The males of the genus *Eristalis*, in the early spring when the females are scarce, behave in the same way; they chase any insect which has a bee-like hum, such as they make themselves, even examining the very dissimilar females of humble-bees. I must add that Gruhl (1924) thinks that such behaviour is entirely due to errors of sight, but I believe, in these cases, sound is equally important.

The auditory organs in flies are not, as a rule, so complex as those of the grasshoppers; Mayer (1874), however, has shown by ingenious experiments that male mosquitoes can, almost certainly, locate the direction from which the trumpeting of the female is proceeding. This power depends on the fact that the hairs on his antennae which point directly towards the female do not vibrate, while the other hairs react, each to the appropriate degree. Gruhl (1924) also reports that noises have an effect on swarms of flies dancing in the air, and Landois (Darwin, 1894,

p. 280) asserts that he was able to draw down swarms of gnats by uttering a particular note.

Hering (1926, p. 190) describes some of the numerous stridulating organs, of the most diverse types, which occur sporadically throughout the Lepidoptera[1]. Where the structures are sexually dimorphic they are usually best developed in the male; Jordan (1921) discusses certain Saturniids, however, in which the female alone possesses these organs. Unfortunately the actual use of these organs in mating seems never to have been described. Many moths, besides an antennal organ that is supposed to perceive sounds, have an elaborate tympanic organ in the abdomen or thorax (Eltringham, 1923; Eggers, 1919). In the moth *Chrysiridia ripheus* Drury, with which Eltringham dealt, the organ is sexually dimorphic. In the majority of moths possessing this organ, no sound has ever been heard; but structurally the resemblance is so great to an organ for reacting to sounds, that it is possible that these insects can emit sounds inaudible to the human ear.

10. GREGARIOUS MATING.

It has already been mentioned that the mating of mayflies is often a social affair. This is equally true in several other groups. In flies, for instance, swarms are frequently seen in many species[2]. Gruhl (1924), in particular, has treated the question of swarming in flies at great length, and brings forward much interesting evidence to show that such flies are mainly orientated by the wind, and that the special type of flight characteristic of a swarm often depends on the strength of the wind. It is important to bear this in mind, in order that special types of flight may not, in some cases, be mistaken for displays.

In certain hymenoptera, *e.g. Bembix nubilipennis* Cress. (Rau, 1918, p. 9) and many species of *Andrena*, both sexes occur together in large numbers for a short time of the year, and in the former case, at least, the habitat is sufficiently prescribed for a definite swarm to be formed.

This gregariousness is probably due to different causes in different cases. It is at least in part due to the narrow limits of the habitat preferred by such species, as has just been suggested in *Bembix*. The species of *Ephemera*, also, never go very far from the water in which they spend the first and longest part of their life history. It is further necessary to explain how it is that vast numbers of individuals of a species manage to synchronise their emergence; this difficulty has been emphasised by the Raus (*loc. cit.*). One morning or evening there may be no sign of a species, while a few hours later it may be present in thousands. Presumably, by some process at present not understood, all the individuals are able to restrain the final act of metamorphosis until some sign shows them that the weather conditions are unusually propitious. In the ants, for example, it is the workers that hold back the eager males and females, with the result that swarming occurs simultaneously

[1] The following papers deal with the production of sound in the Lepidoptera: 90, 164, 176, 202, 217, 389.
[2] The following papers describe the swarming activities of various flies: 3, 7, 74, 101, 155, 186, 285, 342.

in all the nests in a large area, and cross-pairing between individuals from different nests is secured.

When once the swarm is formed, it certainly acts largely as a landmark by which the members of a species find one another for mating. As a general rule, the swarms consist of males only; the females either mate as soon as they approach the males, or they sit in their vicinity, and fly up to pair, perhaps when the sight of the dancing males has sufficiently excited them.

This method of mating must make any sort of selection exceedingly difficult. The female would find it impossible to pick out a choice male from the dancing multitudes, while the male who paired with her would almost certainly owe his success to the probably accidentally determined course that the female took in crossing the swarm.

The synchronous flashing of fireflies and the synchronous chirping of various Orthoptera seem to me to have much in common with the aerial swarms of flies and other insects[1]. The two former phenomena have given rise to a fairly extensive literature, and there is now little doubt that both, in spite of some initial incredulity, really occur, in certain places not uncommonly. Morse (1924), for instance, quotes an observation of Carveth Wells in the Malay Peninsula. "One evening I saw a demonstration of insect organisation which, I believe, it is impossible to explain. It was a beautiful night. The air was full of extraordinary fireflies. About every fifteen minutes these fireflies separated into two armies, one settling on the trees growing on the left bank of the river and the other on the right. Then, when I had decided that the fireflies had gone to bed for the night, the whole army on the left bank gave one big flash in perfect unison, which was immediately answered by another big flash from the right. How these flies managed to keep time absolutely beats me, but they did so though there must have been thousands of them stretching along the riverbanks for a hundred yards or more. The illumination was so strong that the branches of the trees could be seen quite distinctly." Other observations show that a somewhat similar synchronous flashing may occur in South Europe, and North and South America.

In the Orthoptera, Allard (1918) describes the rhythmic chirping of the North American cricket *Cyrtoxypha columbiana* Caudell. Four or five of the males chirped 98 consecutive times, in only eight of which the chirps were not in unison. Out of a total of 870 chirps, divided into fourteen periods, 92·8 per cent. of the chirps were synchronous. Similar behaviour has been recorded in the tree-cricket *Oecanthus niveus* De G., and in the long-horned grasshopper, *Neoconocephalus exiliscanorus* (Davis). Very similar is the periodic upward flight from the surface of water of the swarms of the mayfly *Palingenia papuana* Etn. (Eaton, 1883).

I think that this gregarious use of activities which normally facilitate mating is essentially the same as the swarming of flies, and, in the same way, is probably due to different causes in the various insects concerned. As regards the mechanism of synchronism, Hudson (1918) would have us believe that, in reality, a few con-

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[1] The following papers deal with the synchronous flashing of fireflies: 8, 9, 22, 138, 156, 192, 265, 266, 267, 268, 321, 331, 359, 380, 399. For accounts of synchronous chirping see: 9, 10, 349, 388.

tiguous fireflies, flashing simultaneously, start a wave of luminescent activity, which passes rapidly through all the assembled individuals. The Snyders (1920) have a rather different, physiological explanation. In the absence of any personal observations, I cannot discuss this subject further with any propriety.

It is quite possible that the assemblages of insects occasionally observed on the tops of mountains or gathered round high buildings may be of the same nature as the gregarious mating seen in other species[1]. In both cases a landmark is provided for stray individuals of the species. There is some evidence that certain groups, *e.g.* the flies of the family Oestridae, gather only on high places while other families mainly form the more usual type of swarm.

11. THE USE OF SCENT-ORGANS IN MATING.

When Darwin wrote about Sexual Selection (even in 1894) little had been written of the scent-glands of insects, and there is no reference to them in his book. Fritz Müller's work on the scent-organs of Lepidoptera, which he began in 1877, was not familiar to English entomologists for many years. His collected papers on the subject have now been published in an English translation (Longstaff, 1912, appendix), and, more recently, these glands have been the object of intensive study, being now described in most of the groups of insects, and shown, frequently, to play a dominant part in the processes which lead to mating.

At present it is still amongst the Lepidoptera that these organs are best known[2]. The most diverse types of scent-organs are found in the males, and often in this sex there is a division into a scent-producing gland, and a scent-distributing brush. In some Danaine butterflies the gland is situated on the wing, being covered by modified scales or invaginated into a pocket, while the brush is found near the apex of the abdomen. Lamborn (quoted by Poulton, 1918 *a*) has seen the male wiping his wings with his abdomen, to transfer the scent to the brush. In some species of *Amauris* (Eltringham, 1915) the brush is full of a dust, formed of disintegrated hairs of a special type, which absorbs this scent, and which the male has been seen (Poulton, 1914) to sprinkle on the female in courtship.

In the less specialised forms the gland and the brush form one apparatus, which may be situated on the palpi, legs, wings or abdomen, and is frequently extrusible. These glands and brushes are found to differ considerably amongst allied species (cf. Eltringham, 1915), and in some forms they are different in the two seasonal forms of the insect (Ball, 1914; Dixey, 1920). These male glands are probably always used to excite the female.

The female herself is provided with glands, though these seem to be less diverse in structure and have been less studied. Their effects, however, are much more remarkable. The best known instances occur, of course, amongst the Saturniidae and Lasiocampidae, and some of the most conclusive experiments have been

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[1] For accounts of the assemblage of insects in elevated localities see: 46, 101, 253, 278, 300, 341, 343, 346, 390.
[2] The following papers deal with the scent-organs of Lepidoptera: 29, 30, 31, 32, 77, 78, 82, 83, 84, 85, 99, 111, 112, 116, 117, 119, 131, 157, 164, 176, 179, 197, 213, 227, 228, 229, 230, 257, 302, 307, 308, 311, 364, 379.

performed by Mayer (1900) on a moth, *Callosamia promethea* Drury, belonging to the former family. The dissemination of the scent of the female could be stopped entirely by placing her in an airtight vessel, and, now, even if enclosed by glass, the males would take no notice of her. The males were almost equally attracted by any object on which the female had rested for a time, and also, if the abdomen was removed from the female, it was to this alone that they assembled. When the female was placed in a chamber with two entrances, through one of which air entered, while through the other a current passed out, the males were attracted to the latter only, and were undeterred by powerful chemicals. The distance that males are attracted is probably often exaggerated and, in this connection, it must be remembered that the males of these forms have often a very erratic flight, coursing about in all directions; there may be many of them, therefore, passing through a district where the species is apparently rare or absent[1].

Freiling (1909) and Urbahn (1913) have described the scent-glands of a number of female moths and butterflies. They are frequently situated on the intersegmental membrane of some of the apical segments, sometimes being eversible sacs, as in the silkworm moth. In the case of *Orgyia*, Freiling saw minute drops of liquid, like sweat, on the surface of the everted glands; when the liquid was absorbed by blotting paper and held before a fresh male, it made him behave exactly as if he was in the presence of a female; he fluttered his wings and attempted to copulate with it. Lamborn, also (Poulton, 1912), has observed the extrusion of the scent-sacs in courtship in a female Pierine.

As a general rule the males of those species which assemble to the female have also much the most complex antennae; they may be either branched or have long hairs, and are almost certainly the seat of his scent-perception (see Nieden, 1907).

In a few species the normal activities of the sexes seem to be reversed, the female seeking out the male. This seems to be the case in *Hepialus humuli* L.[2], and also *Biston hirtarius* Cl. (Gillmer, 1922); in the latter species, curiously enough, the male has still bushy antennae, while those of the female are simple. Possibly *Triglochana* (*Aegeriidae*) (Lecerf, 1920) is another example, since the females of this genus have most of the secondary sexual characters peculiar to the males in other members of the family.

The caddis-flies have varied scent-organs in the male which have been described by several authors. One of the common types, resembling that found in many moths, consists in a special development of the male maxillary palps. These differ, in various ways, from those of the female in many families, and, in such forms as *Sericostoma personatum* Spence (Cummings, 1914), the palp is extraordinarily enlarged in the male, densely tufted, and has a glandular internal structure. Müller (1887) noticed a strong smell of vanilla in this species when in copulation. Wesenburg-Lund (1913) has, also, described the mating of *Mystacides nigra* L., in which

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[1] The following papers give accounts of assembling in Lepidoptera: 14, 24, 50, 125, 144, 151, 245, 246, 317, 345.
[2] The following papers deal with the secondary sexual characters and courtship of the Hepialidae: 23, 25, 29, 77, 78, 243, 314, 325, 326, 327.

species the preliminaries take place over the water. The male comes up behind the female, beats on the middle of her abdomen with his long palpi, and the pair fly to the shore, where pairing takes place. In the same genus, Kellogg (1894) has recorded the presence on the wings of the males of modified scales, like those of Lepidopterous scent-patches. Müller (1887), also, mentions a fold, provided with a hair-pencil, in the hind wings of the males of the genera *Halesus*, *Ecclisopteryx* and *Drusus*, which belong to two different families.

Mosely (1919 and 1923) and Eltringham (1919 b) have further described, in the genus *Hydroptila*, remarkable eversible, cephalic scent-organs by whose structure closely allied species can be distinguished.

Amongst the Neuroptera, Withycombe (1922 a) has described the male scent-glands of *Osmylus chrysops* L., and their function in mating. The male plays a passive rôle, sitting still on a leaf, and everting the glands from near the apex of his abdomen, while the females search for their mates. Those who find the male, approach his scent-glands and caress them with their antennae and palpi, till the male withdraws his glands, and courtship is continued in a more normal manner. Eltringham (1926 b) states that many male ant-lions have a scent, and figures an organ in the hind wings, which may be the source of this odour.

In the Diptera, Feuerborn, in a very interesting paper (1922 b), has described the scent-organs that are found in the Psychodidae[1], and the use to which they are put in some of the species. It must be stated that it is very difficult to prove rigidly the exact function of an organ in these small insects; the evidence is mainly circumstantial, but, in view of our knowledge of the behaviour of other groups, it is fairly convincing.

The structures that Feuerborn has discovered can be divided into three main types. Of these, the first are the so-called askoides—tube-like, curving or branching processes arising from some of the apical antennal joints. These askoides are often equally developed in both sexes, but in some forms they are more numerous and branched in the male, especially in those species in which other types of scent-organs are lacking. Feuerborn supposes that these organs, which are connected internally with glandular cells, emit a scent by which the sexes recognise one another. He has shown that sight plays practically no part in the recognition, and there is no other organ to which he can refer the production of the scent, by whose perception the sexes, if sufficiently near, become aware of one another's presence, even if one of them is hidden.

The second type of scent-organ is the so-called "epipterygal organ," an invagination at the base of the wings on each side, surrounded internally with glandular cells, and covered by a fold of the wings. When the sexes perceive one another, if both are in the right state of maturity, each vibrates its wings so as to uncover the organ, liberate its secretion and fan it into the surrounding air. This scent, he supposes, acts as a mutual signal of readiness for mating.

Thirdly, certain species, *e.g. Ulomyia uliginosa* Mg., which he observed in

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[1] The following papers deal with the secondary sexual characters and mating habits of the Psychodidae: 44, 79, 98, 128, 355, 375.

detail, have flexible thoracic processes of various kinds with an eversible scent-producing apparatus at their apex. In *Ulomyia*, the courting male suddenly runs up against the female, face to face, spreading one wing forwards so as to touch one of the females, and bends his thoracic processes forward, so that the everted scent-organs lie on each side of her face. These organs Feuerborn regards as adapted to raise the sexual excitement of the female to a level sufficiently high to admit copulation, which normally occurs immediately after his "embrace." Some species, in addition, have glandular structures in connection with the male claspers, which may play a part in controlling the reactions of the muscles of the female genital orifice.

The preceding account includes by no means all the remarkable secondary sexual and epigamic characters found in the group, and the marked differences between allied species have not been mentioned. For further particulars the reader must be referred to Feuerborn's paper (1922 *b*).

Howlett (1912 and 1915) has also obtained some curious evidence as to the importance of scents in the courtship of flies. He found that the males of the fruit-flies of the genus *Dacus* (*Trypetidae*) are strongly attracted by compounds of the oil eugenol. Thus methyl-eugenol attracts *D.* (*Bactrocera*) *zonatus* Saund., while iso-eugenol attracts *D.* (*B.*) *diversus* Coq. *D.* (*B.*) *ferrugineus* Bezzi is attracted to some extent to both these chemicals, especially the latter. Eugenol itself is quite unattractive to all three, but, by exposing it for some weeks, one male of an entirely new species was captured. There is at present no proof that the males are attracted to these substances because the latter smell like the female, but, since the males alone can be caught in this way and in vast numbers, and since they perform mating antics under the influence of these chemicals, it is more than likely that the female gives out some scent resembling eugenol compounds, at least superficially. The females certainly have a faint aromatic smell, but it is difficult to say whether this is really like eugenol.

The Ceratopogonid midges of the genera *Bezzia* and *Palpomyia* (Edwards, 1920 *c* and 1926 *b*; Hoffmann, 1924) have curious eversible abdominal glands in the females, on one or more segments. Their function is at present unknown, but it is probable, since they are often quite differently constructed in species of the same genus and are confined to one sex, that they are used in mating.

Amongst the Hymenoptera, scent-glands or scents have not often been described except in the social species, where they are probably developed for mutual recognition amongst members of each colony. In the humble-bees, however, the male often has a sweet fragrance, not present in the females. The males, also, have the curious habit of flying in procession from place to place, the spots visited being usually the hollows at the bases of trees, etc.; Sladen (1912) has suggested that they emit a scent for attracting the female to these visiting-places.

Other bees, such as *Prosopis*, or wasps, such as *Clytochrysus sexcinctus* F., have a strong scent, recalling that of lemon verbena, well developed in both sexes; in the bees of the genus *Halictus*, the female is specially fragrant, while in *Andrena denticulata* K., the male has an odour of burnt sugar.

In the bee *Thrinchostoma torridum* Sm. (Morice, 1919) there are modified hairs on a part of the front wing of the male; a structure is thus produced recalling in many details the patches of "androconia" on the wings of butterflies, which, in the case of the latter, have been proved to emit scents. He also records similar structures in the males of the Australian sawflies of the genus *Perga*.

Various authors[1] have recorded in the Ichneumonidae that the females attract the males when the former are still in their pupae, often, when still quite invisible; this power must depend on the emission of some odour. It is probable that scents are produced in some of the other groups of the Hymenoptera, though little seems to be recorded. Adler (1894, p. 142) states that the newly emerged females of Cynipids stand for some time with their ovipositor and adjacent parts extruded; in the sexually propagating generation this attitude is maintained until the male arrives. It is possible that a scent is diffused in this process. Vogel (1921) has shown that the antennae of the male honey-bee have many more sensilli than those of the female or worker: this dimorphism is probably widespread.

Amongst the beetles, epigamic glands seem to be rather uncommon, though Lengerken (1924) describes them in the males of four families. In the Drilidae the degenerate female is very attractive to the males, and the latter can be "assembled" just as in the Saturniid moths. The male has large branched antennae, while those of the female are simple. In the Lymexelonidae (Germer, 1912) the male has either an extraordinarily developed second joint to the maxillary palp or branched antennae. If the palpal joint is removed, he is no longer attracted by the female. According to Lengerken, other beetles with large antennae in the male, belonging to several families, use these organs for finding the female. Hauser (1880) also found that the male cockchafer had more sensilli on his antennae than the female.

Scent-glands are also known in some Orthoptera. Thus many species of cockroach have a dorsal gland in the male[2]. Some controversy has arisen as to its function because it occurs in both sexes in a few forms (*e.g. Blatta*, Minchin, 1890). On the analogy of other insects, it is very likely that the dorsal glands are really always connected with mating. Garman (1891) and Packard (1898) have recorded eversible glands near the apex of the abdomen in certain male crickets. Ramme (1923), in a very interesting paper, shows that all the species of the cockroach genus *Ectobius* are best distinguished by the structure of the dorsal gland and its orifice in the male.

On page 304 of the present paper some account has already been given of the efforts made by male insects to dislodge other males who have succeeded in mating with a female. Further examples are found in the genus *Micropteryx* (Lepidoptera), in the species of which I have noticed that the unpaired males often pay more attention to a copulating pair than to solitary females. Delmas (1926) also records that in the sawfly *Pristiphora conjugata* Dahlb., a male who had just finished copulating, was, when introduced into a vessel containing only males, the subject

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[1] The following papers record the assembling of male Ichneumonidae: 20, 61, 385.
[2] The following papers deal with the dorsal glands of male cockroaches: 214, 215, 256, 259, 316.

of much attention from his companions, who attempted to mate with him. Feuerborn (1922 *b*) has made similar observations amongst the Psychodid flies. It is reasonable to suppose, in view of the great part that scents play in mating, that this behaviour is due to the odour (perhaps produced in the preliminary courtship) that surrounds a mated pair.

The movements of the wings, that have so often been mentioned as part of the preliminaries of copulation, may in many cases help to spread the scent, as is almost certain the case in some species of *Hepialus* (Robson, 1887 *b*).

To sum up then, scent-organs are found to be of very wide and, in many groups, frequent occurrence in insects, playing an essential part in mating. When they occur in the female alone their function is to bring the sexes together, while those peculiar to the male are used, in nearly all cases, to rouse the female to the state in which she is ready to copulate. In the latter case, therefore, their function is exactly similar to that of the various wing-movements and other antics described on some of the previous pages. Hand in hand with the development of female scent-organs goes the development of male perceptor-organs, usually situated in the antennae.

In all these developments there is an extraordinary amount of parallel evolution and convergence. This has been well shown by Fritz Müller (*loc. cit.* p. 638). This is not so surprising, however, as at first sight appears, for though the organs occur in very diverse situations, they are essentially similar in structure, and are displayed, in many cases, by movements common to the courtship of many insects. The organs usually consist of a more or less complex aggregation of glandular cells, a brush composed of hairs or scales developed from the normal type of body covering, the whole apparatus, in many forms, being eversible by the fluid pressure of the body cavity. The eversible structures are often found in parts of the body where there would, in any case, be convulsive movements just before mating.

In spite of the convergence between distantly related forms, the scent-organs of close allies are often quite different, and may provide useful specific characters.

12. THE COYNESS OF THE FEMALE.

In the preceding pages an attempt has been made to set forth the types of secondary sexual characters which occur, and, whenever it has been possible, the meaning of this dimorphism in the life of the animal concerned. Many of the male structures and types of behaviour seem to be used by him for exciting a female, who is less eager than he for copulation, to a state in which she will pair. This presupposes unwillingness on the part of the female, and this "coyness" requires explanation, because it would seem at first sight that the quicker mating was accomplished, the better it would be for the species. Amongst birds, Huxley (1923) has suggested, with some probability, that the female is coy in order that copulation may not take place so often as to exhaust the individuals or expose them to a dangerous extent to the attacks of their predaceous enemies. This does not, of course, tell us what physiological process leads to coyness in the female; it only suggests that such a process would be beneficial. In insects the term "overcopulation" requires better definition. If the males are more abundant than the

females, when the time for mating arrives, then, besides the opportunity for choice amongst several possible mates given to the females, there are, in addition, three dangers to which the species may be exposed. First, the females might be willing to copulate too often and would have the opportunity to do so. Secondly, the vulnerable copulated pairs might be harassed too much by unmated males, drawing the attention of predaceous enemies, perhaps, to pairs who would otherwise have escaped notice. Thirdly, the males might pester females already fertilised who ought to be either nesting or ovipositing.

Copulating too often, besides exhausting the individuals, might also be supposed to expose the relatively vulnerable conjoined pairs to their enemies. No doubt in animals such as the higher vertebrates, that have flexible instincts, copulation might, in certain circumstances, actually be so frequent as to be harmful, but it is difficult to believe this in insects, whose behaviour is so largely governed by relatively invariable instincts that lead them to act in a way beneficial to the species. Again, during the actual process of copulation insects are probably less exposed to their enemies than during the preliminaries, since the pair are often motionless and sometimes in hiding (Poulton, 1904 a). Coyness would not stop males pestering the females, even when these were unwilling; and, further, there is at present very little positive evidence for attacks made on courting or mated pairs of insects.

The second danger does not seem to be very serious in practice, but the third is a real one, as any field-observer knows, and certain habits and structures seem to have been developed partly as a protection. It is further probable that some of the behaviour resembling coyness in the female or display in the male may be due to special needs, such as that of the female for animal food, or that of the male to adopt a special attitude in copulation, owing to the structure of his genitalia. These special factors influencing behaviour before and during mating will now be considered.

13. THE EVIDENCE WITH REGARD TO OVER-COPULATION.

It is very difficult to get accurate information concerning the relative abundance of the sexes, and such data are not usually easy to interpret. The ratio at emergence seems to be equal in the majority of cases, with unexplained anomalies in particular species. Thus in breeding *Stegomyia* (Young, 1922) the males are always more abundant than the females, while in other species, *e.g. Alysia manducator* Pz. (Hymenoptera) (Alston, 1920), the ratio seems to vary. In species which are able to reproduce parthenogenetically there may be a great excess of females. The sex-ratio at emergence, however, has in most species little bearing on the amount of actual and attempted copulation. This will depend on various factors, such as the relative times of emergence of the sexes; the existence of monogamy or promiscuity in the species; the duration of copulation, especially in short-lived forms; the presence or absence of a relation between the sexes which may be called marriage; and the reaction of each sex to external conditions

As a general rule the males emerge before the females, *e.g.* in nearly all British

bees and wasps, many Lepidoptera (cf. Petersen, 1892), many flies and Ephemerids. This is a commonplace to any constant field-observer, and it is supported by a certain amount of breeding (various Lepidoptera, Petersen, 1892; *Stegomyia*, Gordon, 1922; *Scleroderma*, Wheeler, 1924 *a*; *Anechura*, Fraustorfer, 1921). Gruhl (1924) notes that, in the Diptera, the sex which emerges first is usually the one that is most abundant in the field. He substantiates this, to some extent, by examples from the Empididae, in which family some species have one sex, some the other, both the first to appear and the most abundant. There is no entirely satisfactory method of obtaining these facts in all cases, for breeding in artificial conditions is not always satisfactory, especially if there is a high mortality, while field-observations are often deceptive owing to the different habits of the sexes, which sometimes make the male more conspicuous, as in *Apatura iris* L., or apparently rare, as in the Tabanid flies.

Petersen (1892) shows, in the Lepidoptera, that the eggs of one batch develop at an approximately equal rate, and even in the pupa, development proceeds very slowly till near the end of that period. A short time before emergence morphogenesis suddenly accelerates, and the males hurry on in front of the females. The result, which he regards as an adaptation, is that members of the same batch of eggs rarely mate with one another. At the present time, however, it is difficult to believe in a teleological explanation of an unknown physiological process, without more definite evidence. Petersen further points out that this early emergence of the males cannot be very significant in Sexual Selection, because it often leads to males being quite tattered before the females appear. It might, nevertheless, lead to over-copulation.

In some species the female is not always so soon ready for mating after emergence as the male. This point will be discussed later; the result is to increase the functional preponderance of the males.

Again, in some species a female will only admit pairing once, while the male may pair more often. This seems to be the case in the silkworm moth (Michael, 1923) and in some Psychodids (Feuerborn, 1922 *b*). In the latter insects the female also entirely loses her attractiveness to males after mating, probably owing to the fact that she no longer emits her scent. The reliable data on this point are not very numerous, but it is well known that many other species pair more than once, often with several individuals. In such an example as the silkworm, of course, the males become, in effect, much more numerous, provided they do not die very soon after mating.

The duration of copulation may not be very important in long-lived species; but in short-lived ones, if the males are more numerous than the females, the complete withdrawal of a pair from struggle for mates would intensify the competition, since the loss of one female would alter the sex-ratio of active individuals more than the loss of one male. In any species the duration of copulation might be an important factor at the beginning of the season, when females were still scarce. There is not much information on this point; Gruhl (1924) has summarised the data for the Diptera, and in this group, generally speaking, the time occupied is

short, though variable. In *Cylindrotoma*, however, the pairs remain in copula for 17–24 hours. In other species much shorter times may have an effect, if the weather conditions governing mating have narrow limits. Unfortunately it is scarcely possible to find a species about which enough is known to correlate all the facts of the life history. In the Brimstone butterfly, *Rhodocera rhamni* L., Labitte (1919) found that a pair remained in copula for a week, but this may have been exceptional, since the insects were transferred to captivity. In moths, mating often lasts twenty-four hours, but the greater part of that time is daylight, when pairing would not, in any case, be initiated. A rather similar state of affairs seems to exist in the Lampyrids, according to the account given by Hess (1920). Probably in these forms copulation is effected in many individuals at the beginning of the flight-period (*i.e.* twilight), and the pair remain in copula till after the flight has ceased. If there was a preponderance of males this would have an effect on the competition for mates; but it is difficult to estimate the effective sex-ratio of Lampyrids owing to the differences in the habits of the sexes. In conclusion, then, in most species copulation probably does not last long enough for its duration to be an important factor; in a few forms, however, copulation for a long period, or for a period long compared with that in which mating can occur at all, may make a significant difference to the effective preponderance of the males.

Similar in effect to a lengthy copulation-period is the institution of what may be termed marriage, found in various groups of insects. Thus the Peckhams (1905) and Kennedy (1838) have seen the male of species of *Trypoxylon* keeping guard over the nest, with his head closing the entrance. On the return of the female he flies to meet her and she carries him back into the nest, seated on her back. It does not seem to be recorded how often copulation occurs in this genus, or whether the female only copulates with the male who guards her nest. The occupation of one nest by a pair of insects has also been recorded by Fabre (1897) and other observers in various Lamellicorn beetles, and by Gerhardt (1913) in crickets; in these cases, probably, the female would only mate with her "husband," so that there is complete monogamy. The behaviour of the fly, *Ctenophora atrata* L., according to Stein's account (1920), would appear to be somewhat analogous. In the beetle *Rhytirrhinus*, also, Dumont (1920) found that the male would stay for nearly a month on the back of one female.

As regards the effect of external conditions, the data are even more scanty. Carpenter (1913 *b*) found that in the Tsetse, *Glossina fuscipes* Newst. (*palpalis*), the female is much more easily killed by drought and high temperature than the | male, and that the effective sex-ratio may be governed entirely by this factor. The differences in habits of the sexes must expose them to a different degree to the attacks of enemies, *e.g.* the Bibios caught by Empidid flies are nearly always males, a circumstance almost certainly mainly due to the special type of flight in the males of the former genus. Walker (1912) records that in some districts the females of the dragonflies of the genus *Aeshna* are much rarer than the males; this is mainly due to frogs catching a large number when they are ovipositing, and, higher in the mountains where frogs do not occur, the disparity is not found. Some insect enemies take

only one, or mainly one, sex, *e.g.* various species of *Crabro* (Hamm and Richards, 1926). In some moths, on the other hand, Rau (1912) finds that there are differences in potential longevity, which may be accentuated by continued celibacy; Glaser (1923), also, finds that the females of the housefly generally live longer than the males. Differential longevity will alter the sex-ratio as the season advances.

Although accurate records are not very frequent, it seems to be the case, then, that, of the individuals prepared to copulate, the males are often much more numerous than the females. The mobbing of females and of copulated pairs has often been noticed (Mosely, 1899; examples in the present paper). This must make the species conspicuous, and must hinder the female in her oviposition. I have seen the females of the sand-wasp, *Cerceris arenaria* L., who were storing their nests and long past the mating period, mobbed by males, who clung to their backs, so that the female could not enter her burrow: she would scrape them off her back by crawling between two pine-needles. Such delay must expose the nest to parasites and must waste the short allowance of fine weather that the female receives in England.

Further, in certain insects (*e.g. Samia cecropia* L., Rau, 1912: *Dendrolimus pini* L., Eckstein, 1911) the female usually, or always, pairs on one occasion only; in the first-named moth at least, the males nevertheless survive for some time. It is difficult to obtain reliable records of insects dying after one mating only, as is often popularly reported; but in some social insects (bees and wasps) the male genitalia are actually torn out of his body when the insects separate, so that he necessarily dies. In other species, considered later (p. 331), the female eats the male during or just after copulation.

The most curious protection of the female, however, is the production by the hypertrophied accessory glands of the male, of a seal to the female sexual orifice, making another mating impossible. This structure is known in butterflies as the "sphragis" (Eltringham, 1925 *c*; Bryk, 1919). It has been developed in *Parnassius*, *Amauris*, and *Acraea* (and some allied genera), that is in forms belonging to two different families. An essentially similar structure is found in the water-beetles *Dytiscus* and *Cybister* (summary in Wesenburg-Lund, 1913). This does not stop the males from paying attentions to the females, but it may discourage them sooner. It is probable that there is still much more to be discovered as to the significance of the sphragis.

14. THE FOOD OF THE FEMALE.

The eggs laid by the female are, of course, much bulkier than the sperm ejaculated by a male, and there is some evidence that the female has special food requirements in consequence. In the honey-bee, the queen-producing larvae, as has long been known, receive a food richer in proteins than do the other castes (von Planta, 1888). In the stingless bees (Wheeler, 1924 *a*) the female is not so favoured, and, probably as a consequence, she requires some weeks after emergence to mature her eggs.

In predaceous insects, as a general rule, the female is much more voracious than the male. Edwards (1920 *b*) records this in the Ceratopogonidae. Hess (1920) found the same in the Lampyrid *Photuris pennsylvanica* De G., whose female eats chiefly

the males of other species of fireflies, to which she is probably attracted by their flashing. Hess never saw the male *Photuris* eat at all, but he supposes that they must, since they live for two or three weeks. In *Luciola*, however, Emery (1884) finds that the gut of the male is functionless, and distended with air. In Asilid flies the females undoubtedly eat more than the males. Thus Poulton (1906) found that of the 207 Asilids, of determined sex, he recorded with prey, 160 were females. I believe, from my own observations, that in the predaceous sawflies (genus *Tenthredo*) the females eat much more animal food than the males. In those Empidid flies in which the prey is not connected with courtship, the females seem to be the more voracious, and, at least in the *Xanthempis*-group, it is that sex alone which preys on other insects.

The facts with regard to blood-sucking flies are widely known. In some Liponeuridae, in some Ceratopogonidae, in the Simuliidae, in most Culicidae, in some Psychodidae, in the Tabanidae, and certain Leptidae, only the female sucks vertebrate blood, the males taking honey, or, in some cases, not feeding at all. In the mosquito *Stegomyia*, Gordon (1922) has proved that a female who has not received her blood-meal, lays eggs which do not hatch. Similarly Glaser (1923) has shown that in *Musca domestica* L. and in *Stomoxys calcitrans* L. certain fairly specific foods are necessary before any eggs can be laid at all. It is quite possible that the whole evolution of such insect groups as the fleas (Siphonaptera) was originally initiated by this need; some of the Muscidae (*e.g. Stomoxys* and *Glossina*) and the Hippoboscidae, in which both sexes may suck blood, exhibit, perhaps, an intermediate type of behaviour.

In certain cases the blood of insects is required instead of that of vertebrates, *e.g.* in *Forcipomyia* (Saunders, 1924; Edwards, 1923) and *Hemerobius* spp. (Withycombe, 1922 *a*); in both these genera there is some experimental evidence that this food is necessary for the laying of fertile eggs.

Some of the most remarkable developments of insect courtship seem to be mainly due to the need of animal food in the female. In some forms, such as various Orthoptera (Gerhardt, 1913 and 1914) and Neuroptera (Withycombe, 1922 *a*), the male introduces his sperm in the form of a spermatophore, part of which protrudes from the female orifice after pairing. The female always takes advantage of this to eat most of the spermatophore, and in *Osmylus* (Withycombe, 1922 *a*) it is probable that the male remains gripping the female after copulation, in order that she may not eat up the spermatophore before any sperm has left it. In the Locustid grasshoppers[1] (Boldyrev, 1913; Gerhardt, *loc. cit.*) one lobe of the spermatophore, the so-called spermatophylax, is devoted to satisfying the female's appetite; by the time it has been eaten, the sperm has passed into the spermatheca. This type of behaviour has been still further specialised in the tree-crickets, *Oecanthus*[2], whose habits have now been observed in both America and Europe. The male has a curious pit in his metanotum, containing tufts of hairs and well

[1] The following papers describe the mating habits of grasshoppers: 40, 58, 130, 140, 141, 142, 298, 374.
[2] The following papers describe the glands of the male and the mating habits of *Oecanthus*: 122, 132, 165, 184, 200, 349.

supplied with glands (von Englehardt, 1914). The male approaches the female, uttering a special song, and eventually she climbs on to his back and starts eating the secretion of the gland. After several minutes copulation is effected, but only lasts a short time, the female staying on the male's back both during and for some time after it. As soon as she leaves the male for good, she eats up all that is left of the spermatophore. Boldyrev (1913) has shown that if the female be removed from the gland immediately after copulation, she will eat the whole of the spermatophore before the sperm has left it, and he regards the gland mainly as a protection for the sperm. It is almost certainly also a lure to the female as well as a source of food. Fulton (1915) has found that not only females, but also nymphs, take advantage of the metanotal gland of the male.

In certain flies the male regurgitates a drop of food for the female before or during mating. Piersol (1907) has described the habits of *Rivellia boscii* R-D. When the male has succeeded in mounting on the back of his mate, copulation soon takes place, provided the female is willing to mate at all. During copulation there are periods of excitement in which the wings are moved more rapidly, and each fly quickly extends its mouth-parts several times; in a few seconds at the end of the male's proboscis a globule of liquid food can be seen, which is passed over to and eaten by the female. This is repeated many times during copulation. Wheeler (1924 b) has recorded very similar behaviour in another fly, *Cardicephala myrmex* Schin.

In the scorpion-flies, *Panorpa*, there is a great sexual dimorphism in the salivary glands, those of the males being very large. The male, just before pairing, secretes saliva, which hardens into little globules, on to the leaf on which the pair are sitting, and while the female makes a meal, he seizes the end of her abdomen with his genitalia and copulates (Summary in Stitz, 1926).

These examples lead naturally to the behaviour of the Empidid flies, in which the male hands over to the female, at the moment of mating, an insect prey caught for the purpose. An outline of this ceremony has already been given on page 309. I think there can be little doubt that the primary function of the prey is to supply the female with necessary food; the food is provided by the male because, in all probability, certain features of the genitalia (see p. 322 *et seq.*) require that he should be ventral to the female at the moment of pairing. The habits of Empidids are evidently very specialised, and the eating, in some species of *Hilara*, has probably become a ritual in which the female goes through the motions of eating a prey provided by the male, even when he gives her only an empty web. This ritual behaviour is not so surprising when we remember that much of an insect's life history depends, probably, on chains of reflexes, each one of which conditions the following one, and none of which can be omitted without interfering with all the succeeding reactions. This interpretation is in disagreement with that of Gruhl (1924), who has made many more observations on these flies than I have. He would regard the prey as merely a lure to attract the female; in such species as *Hilara maura* F., in which a web, which may or may not contain prey, is used, he would suppose that the prey is left out only when, owing to a local scarcity, the males have failed to find anything

suitable. He does not explain why insect-prey should be so successful a lure. I think, however, that additional light is thrown on the Empididae by comparing their habits with other groups.

One further observation, made by Gruhl on *Hilara maura* F., deserves comment. He found that solitary unmated males seemed usually to have their web empty of prey, while a high proportion of the mated males had a victim in the web. It would appear that females mated more often with males who had a real meal to offer them, selection here thus acting, apparently, against the direction in which it is probable that Empidid evolution has proceeded.

Finally, in a number of insects, the male is eaten by the female during or after mating. Fabre has recorded this in the beetle *Carabus auratus* L. (1910), and in *Mantis religiosa* L.[1] (1897). Howard (1886) gives an extraordinary account of *Stagmomantis carolina* L. A male and female were put together in a vessel and the latter soon attacked the male. When she had eaten one front leg and one eye, the male realised that he was dealing with a female, and began frantically trying to copulate. The female continued eating him, and when she had consumed his head and most of his thorax suddenly opened her genitalia and copulation was effected. It lasted about four hours, the male showing signs of life for some time; next morning he had been completely devoured.

Goetghebuer (1914) has also recorded that the female of the Ceratopogonid fly, *Johannsenomyia nitida* Mcq., eats the male after copulation; such females are found with the male genitalia still clinging to them, all the rest of him having gone. Edwards (1920 *b*) describes similar behaviour in *Serromyia femorata* F., the pair copulating belly to belly with their mouth-parts joined; at the end of pairing the male's juices are sucked out through his mouth.

Solowiow (1924) also mentions the rapacity of the female in *Atropos pulsatoria* L. (Psocidae); she eats both males and young of her own species.

It is possible that in the Mantids a reduction in the number of males is also useful because the female has to spend much time and care in the construction of the egg-case; importunate males might easily damage the case when it was still soft.

In the *Johannsenomyia*, also, there is a specialised method of oviposition; the fly hovers over the water of a stream, gradually extruding a long, gelatinous ribbon of eggs (Hamm, 1919).

Certain insects, especially males, are well known never to take food at all, this asceticism being usually associated with atrophy of the mouth-parts and gut (Lymantriidae and Saturniidae, cf. Haettich, 1907; *Ephemeridae*), or gut alone (certain Psychodidae, Feuerborn, 1922 *b*; *Luciola*, Emery, 1884). There is no doubt that these losses are correlated in a general way with special means of enabling the sexes to find one another (usually very efficient scent-organs, but in the Ephemerids exact coincidence in time of emergence combined with localised habitat) and also frequently with a very short life in the adult.

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[1] The following papers describe the cannibalism of Mantids: 123, 185, 313, 315, 318, 324.

15. THE MECHANICAL RELATIONS OF THE MALE AND FEMALE GENITALIA.

The problems involved here have, at present, been very little explored, so that only a very preliminary account can be given. In writing on this subject, I am very deeply indebted to the papers by Lamb (1922) and Feuerborn (1922 a). Owing to various circumstances, it is convenient to deal first with the Diptera alone. In a normal fly, such as *Thaumastoptera calceata* Mik., described and figured by Shuwen Liang (1925), the anus is morphologically dorsal to the genital orifice. Thus, if a line were drawn from the dorsal surface of the base of the abdomen, going round the tip of the abdomen, and back to the base on the ventral surface, such a line would pass across, first the anus, then the genital orifice. This condition is fulfilled, as far as is known, in all female insects, and most males, with certain very interesting exceptions. Snodgrass (1902) showed that in the Asilid genera, *Laphria* and *Dasyllis*, the anus and the genital opening are in reversed positions; this is due to the fact that the last few segments, which are fused together to form the "hypopygium" on which these two orifices are situated, have been twisted through an angle of 180° about the long axis of the abdomen. More recently, Edwards (1920 *a* and 1924), Christophers (1915), and Feuerborn (1922 *a*) have found the same conditions in all the Culicidae (including *Dixa*), in the Eriopterine Tipulids, and all the Psychodidae; Lindner (1923) records it in *Diadocidia* (Mycetophilidae), while I have found that the abdomen of *Bombylius discolor* Mikan (though not of *B. major* L.) is similarly affected. Such male flies are said to have a "hypopygium inversum." Further, Feuerborn has shown, by interpreting the anatomical study of Bruel, that in *Calliphora*, the hypopygium has been twisted through an angle of 360°, so that the relative positions of the anus and the genital orifice are again apparently normal, a condition which Feuerborn terms "hypopygium circumversum." The twist, however, can be detected, partly in the asymmetry of some of the sclerites just preceding the hypopygium, and especially because the Ductus ejaculatorius has become looped right round the gut. I have been able to verify this condition myself in *Calliphora*, and Minchin (1905) and Tulloch (1906) have described it in *Glossina* and *Stomoxys*, respectively[1].

A twist, leading to the same end result, has also occurred in all Syrphidae; this has been discussed by Lamb (1922), who did not, however, at that date realise the extent of the torsion. Metcalf (1921), also, has figured the genitalia of many North American species of this family, but he does not discuss the torsion which he figures. As a matter of fact, there appear to have been two twists of 90°, and one of 180°, each between different segments, and resulting in a twist of 360° in all; since the torsion is in several stages, the asymmetry is obvious externally. I have found a condition somewhat intermediate between the Syrphidae and *Calliphora*, in species of *Ernestia* (Tachinidae), and it is, as a matter of fact, practically certain that the hypopygium has, in one way or another, received a twist of 360° in all Muscidae s.l.

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[1] Cf. also W. Petzold, 1927, "Bau und Funktion des Hypopygiums bei den Tachinen, unter besonderer Berücksichtigung der Kieferneulentachine (*Ernestia rudis* Fall.)," *Jenaische Zeitschr. f. Naturwiss.* **63**, 1, 1–49.

(*i.e.* including the Acalyptrates). It is very significant that the torsion has taken place between different segments and in some cases in different directions, in the families concerned; other considerations, also, make it almost certain that this torsion has taken place independently at least nine times in the flies alone, while there are, no doubt, more examples to be found in some of the species of the Empididae, Dolichopodidae, Phoridae and other families.

The effect of this torsion on copulation requires careful consideration. Certain terms Lamb has coined are convenient. If we imagine two insects to be pairing tail to tail, then the dorsal surface of the male penis will come into contact with the dorsal surface of the female vagina, a state which Lamb calls "direct correlation"; while if the male be seated on the back of the female, and bends the tip of his abdomen underneath him to copulate, then the dorsal surface of the penis will touch the ventral surface of the vagina, that is "inverse correlation." In some Coleoptera it has been shown that the male penis fits the female vagina so exactly that it would be impossible for it when once inserted to twist within the vagina (Harnisch, 1915, see Fig. 43, p. 49). No such intimate correlation has yet been shown in flies, and in *Drosophila* (Nonidez, 1920) it evidently does not occur; yet the penis is often bilaterally not radially symmetrical, a circumstance which would hinder any twisting within the vagina, and there are good grounds for believing that such twisting never occurs; if a twist is necessary it always takes place between the hypopygium and the abdomen or in the abdomen itself. Thus it is evident that direct correlation, when once established in the course of mating, cannot be converted into inverse. I believe that in flies the correlation is always inverse, and that if this is admitted all the known facts can be reduced to a common basis.

There is a further term defined by Lamb, the "pose," or attitude adopted by the sexes at the instant at which the penis is inserted into the vagina. There are two radically different poses, from which all the others can be derived. First, the insects may be tail to tail; modifications of this pose are seen when the insects are belly to belly, or when the male is on the back of the female, upside down with his feet in the air. These modifications could be illustrated by taking a pair already firmly joined by their genitalia, and either lifting the male up and laying him on the back of the female, or doubling them up, so that they came to be belly to belly with their heads in the same direction (see Figures, p. 337). The original tail to tail pose Lamb calls the "linear pose" (I.P.); in this pose the hypopygium must be already twisted through 180°, if inverse correlation is to be maintained.

Secondly, the male might be on the top of the female; this can be modified in such a way that the male lies on his back, joined to the female by his genitalia only; if the male be further pulled round he will come to be under the female; in the extreme modification the male is pulled up on one side of the female, so that the greater part of his abdomen is dorsal to her, but the tip of his abdomen passes under hers (see Figures, p. 337). When the male is on top, Lamb calls the pose the "male vertical pose" (MVP.), while if the female is the upper member of the pair, it is the "female vertical pose" (FVP.). The pose in which the tip of the male abdomen only is under the female may be called the "false MVP." In nature, of

course, a male does not have to go through all these stages to reach the false MVP., he merely jumps on to the back of the female and passes the tip of his abdomen down on one side of hers to grip her genitalia from beneath. These poses are only spoken of as modifications of one another, because in all of them the correlation is inverse, provided the hypopygium is either normal or has been twisted through 360°.

As regards correlation there is no difference between FVP. and MVP., but there is a difference in the condition of the female anus. In an insect with a normal hypopygium, copulating in the MVP., the anus of the female is completely closed by the adpressed ventral surface of the male; in the FVP., on the other hand, the male anus would be pressed against the female's venter, while the female would have a free anus. In the false MVP. the male would be able to grip the female, as in the normal pose, but the female would have a free anus.

Finally, Lamb calls the attitude adopted by the pair in the later stages of copulation the "position." The position need not necessarily differ from the pose, but in many insects, when once union has been effected, the male alters his attitude; since, however, his hypopygium is firmly fixed to the female abdomen, he can only change from the pose to the position by twisting his abdomen, and the original pose can always be detected by examining the orientation of his hypopygium. Thus in some Tipulids, e.g. Limnobia (Gruhl, 1924), the male retains the MVP. throughout copulation, unless the pair take to wing, in which case the male trails behind, presumably either lying on his back, or else twisting his abdomen through 180°. In some of the other Tipulids (e.g. Tipula), or in Ptychoptera (Tonnoir, 1919), what may be called a "false linear pose" is adopted. The insects are tail to tail, but the abdomen of the male is already twisted through 180°, so that at its apex the ventral side is uppermost and inverse correlation.is maintained.

With these definitions, we may proceed to the consideration of what takes place in those insects whose pairing is recorded. We may take, first, forms with a simple hypopygium. The pairing of Tipula, Limnobia and Ptychoptera has already been described. From the observations of Stein (1920), Ctenophora atrata L. would appear to copulate in a modification of the MVP., in which the insects are standing side by side instead of the male actually sitting on the female; if they wish to move about, they take up a false linear position. Alexander (1920) describes the mating of Eriocera longicornis (Walk.) which takes place in the air; apparently the male is in the MVP., but the female trails beneath him, attached only by the genitalia.

In the Chironomidae accurate descriptions of mating have not, apparently, been published; Gruhl (1924) states that the position is tail to tail, but very likely he overlooked the twist in the male abdomen.

In the Tabanidae, Pérez (1911) and Gruhl (1924) record the MVP.; in the final position the male comes to lie on his back, being connected with the female by his genitalia alone, and hanging quite passively in the air. The hypopygium is almost certainly normal.

In those Dolichopodidae observed by Gruhl, the male always adopted the MVP.,

sometimes slightly modified as in *Neurigona*; the hypopygium of the forms he observed is normal[1].

In the Asilidae the best observations are those of Melin (1923); the pose seems to be usually the false MVP., and he notes that the male's abdomen may pass on either the right or the left to get beneath the female's. Sometimes the male manages to twist round afterwards so that the linear position appears to be adopted (*e.g. Lasiopogon cinctus* F.), but I suspect that Melin may have failed to notice the twist in the abdomen, which ought to be visible just as in *Tipula*, etc. Gruhl (1924) also records the tail to tail position in some species, but his details of the relations of the two insects are insufficient. Melin (1923) describes *Leptogaster cylindrica* Deg. mating with the female hanging from a grass stem, the male hanging from her attached by his genitalia alone; his back faced in the same direction as the venter of the female, so he was in a modified MVP. position. Mr A. H. Hamm tells me that he has seen *Asilus crabroniformis* L. flying in this position also; this is curious, since in this species Melin records the false MVP., so that if the male manages to twist round so as to be tail to tail and yet be on his back, his genitalia must be subject to a very great strain. I think we may say in these forms that, except certain species with an inverted hypopygium (Laphriinae), the mating pose is probably usually false MVP., and the position is either the same or some modification of it, in which the condition of the abdomen has not yet been sufficiently accurately described.

The Empididae, at any rate of the genus *Empis*, certainly have a normal hypopygium. I have dissected a male of *E. tessellata* F., and I find that the genitalia are quite symmetrical and the ductus is not coiled round the gut. Gruhl has made very careful observations of the pairing of many species, and he finds that the pose is invariably FVP. At the instant of mating, the flies, which always pair in the air, fall down a short distance; this is due, in my opinion, to the fact that the position is false MVP., and the male to reach this position has to pull himself up on one side of the female, making it impossible for her to use her wings just for that moment. In *Empis livida* L. I have examined pairs in copula at rest and find that they are in the false MVP.

Secondly, there are the species with the inverted hypopygium. In all the Culicidae, the pose is either the linear one, or else the two insects are belly to belly, a modification of the LP. (Howard, 1912; Gruhl, 1924). Lamb suggests that it is very difficult to imagine how a male mosquito could fly upside down as would apparently be necessary if the insects paired belly to belly in the air; I think this might easily be done if both insects flew nearly perpendicularly. They would then have their ventral surfaces apposed without flying upside down.

As has already been mentioned, Snodgrass (1902) has shown that the hypopygium of some of the Laphriinae is inverted; I have verified this condition in *Laphria flava* L., and it is evident from Melin's statement (1923, p. 5) that all the Laphriinae he examined lacked the usual anal lamellae, that these forms too are in the same condition. Melin (1923) and Gruhl (1924) have observed the mating

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[1] It is possible that in this family the hypopygium has been twisted through 360°; the matter requires further investigation.

of various species of *Laphria* and they were always found in the linear position, and doubtless the pose is linear also.

Gruhl (1924) describes the mating of *Bombylius venosus* Mikan which takes place in the air in the linear pose. Although I have not been able to dissect that species, I find an inverted hypopygium in *B. discolor* Mikan; in *B. major* L., however, it is normal. I think we may conclude that the hypopygium of *B. venosus* is alsoi nverse, while *B. major* L. probably copulates in the MVP., or some modification of it.

Goetghebuer (1914) and Edwards (1920 *b*) describe the pairing of two Ceratopogonidae; the pose in the latter case was belly to belly, and in the former, if not identical, at any rate some modification of the linear one. These flies have an inverse hypopygium.

In the Eriopterine Tipulids (*e.g. Ormosia*), from my observations, and in the Psychodidae, from those of Feuerborn (1922 *b*), the pose is always linear; in some Psychodids there is a modification in which the insects stand side by side, facing the same way, and bend the tips of their abdomens sideways, towards one another.

Positions adopted by flies in pairing.

Type of fly	Pose	Position	Female anus
Hypopygium normal.			
Limnobia	MVP.	MVP.	Not free
Ctenophora atrata L.	MVP. (modified)	False LP.	Free*
Tipula	(MVP. or false LP.)	False LP.	Free*
Ptychoptera	False LP.	False LP.	Free*
Tabanidae	MVP.	MVP. male hanging back	Free*
Dolichopodidae[1]	MVP.	MVP.	Not free
Asilidae (most species)	False MVP. or FVP.	False MVP. or FVP.; sometimes apparently false linear	Free
Empididae	FVP.	False MVP.	Free*
			Free
Hypopygium inversum.			
Culicidae	LP. or modified LP.	LP. or modified LP.	Free*
(*Johannsenomyia*)[2]	?	Modified LP.	Free*
(*Serromyia*)[2]	I.P. (belly to belly)	LP. (belly to belly)	Free
Laphriinae	LP.	LP.	Free*
(*Bombylius venosus* Mikan)	LP.	LP.	Free*
Ormosia	LP.	LP.	Free*
Psychodidae	LP.	LP.	Free*
Hypopygium circumversum.			
Syrphidae	MVP.	MVP.	Not free
Muscidae (s. l.)	MVP.	MVP.	Not free

[1] See footnote, p. 335.

[2] If one compares the hypopygium of an ordinary Chironomid, *e.g. Tanytarsus* (figured by Goetghebuer, 1922), with that of any Ceratopogonid (*e.g.* spp. figured by Goetghebuer, 1922, or Kieffer, 1925), it can be seen that the so-called dorsal lamella of *Tanytarsus*, etc., is ventral in all the Ceratopogonids, so probably all members of the latter sub-family have an inverted hypopygium. Since the above note was written, Edwards (1926 *b*) has shown that the hypopygium is inverted in all the genera allied to *Palpomyia*, which include amongst them the two species above whose mating position is known. Mr L. G. Saunders tells me that in *Forcipomyia* and related forms the hypopygium is normal, so that we may expect to find another position for mating in these species.
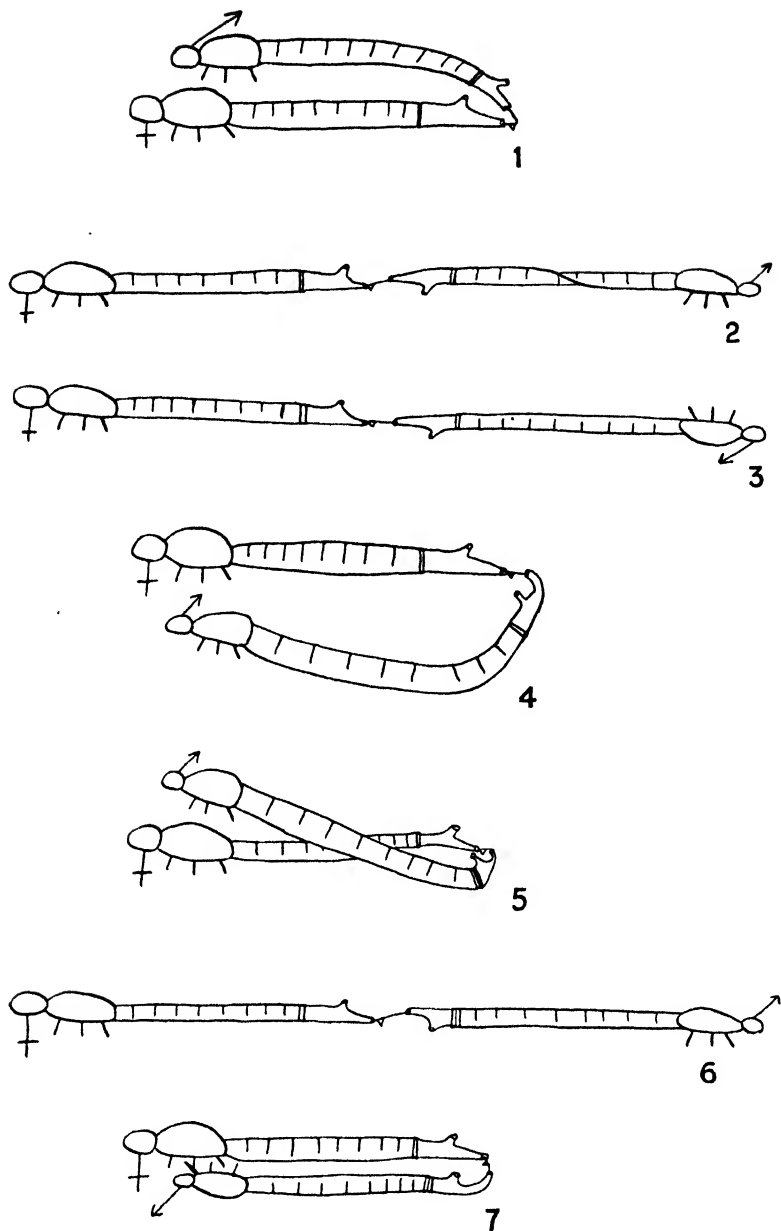
Fig. 1. The "male vertical pose" with a normal hypopygium—*Limnobia.*

Fig. 2. The "false linear pose" with a normal hypopygium—*Tipula.*

Fig. 3. The MVP., with the male lying on his back, joined to the female by his genitalia only—*Tabanus.*

Fig. 4. The "female vertical pose" with a normal hypopygium—*Empis.*

Fig. 5. The "false male vertical position" with a normal hypopygium—*Empis.*

Fig. 6. The "linear pose" with an inverted hypopygium—*Ormosia.*

Fig. 7. The LP., with the insects belly to belly, hypopygium inverted—*Culicidae.*

N.B. The details of the segmentation of the abdomen in the various species have not been indicated. A small triangle has been drawn on the true dorsal surface of the male's penis.

Finally, all the very numerous species in which there is a hypopygium circum-versum, pairing takes place in the MVP. or in some slight modification of it. The only exception with which I am acquainted is the account given by Pérez (1911) of *Chloria demandata* F. His observations seem to show a linear pose, but Lindner's later account of the same species (quoted by Gruhl) definitely records the MVP. I have examined the genitalia of *C. demandata* F. (owing to the kindness of Mr A. H. Hamm, who gave me a specimen) and I find that the hypopygium is certainly "circumversum," but the penis is extraordinarily twisted, and longer than all the rest of the genitalia put together; it is possible that the structure of the penis results in a total twist of 540°, but fresh observations on this fly are needed. (Hendel, 1913, has figured a very similar penis in *Platystoma*.)

These facts can be reduced to the above table in which it will be seen that the pose combined with the condition of the hypopygium always result in inverse correlation. In some species part of the evidence only is available, and I have in these cases put in brackets the state of affairs I believe future observation will show to exist.

A partial definition has already been given of the condition in the female described as "free anus." In one type, when the hypopygium is normal, and the FVP. or false MVP. is adopted, the female anus projects over both the copulating genitalia. In the other, when the insects are in the linear pose, the female anus still projects over the genitalia, though any excreta ejected during copulation would probably fall on to the male genitalia. This second condition is marked with an asterisk.

When we try to bring the other groups of insects into line with the Diptera, the necessary observations are often lacking; what is known may be dealt with briefly.

Without entering into the question of the phylogeny of the orders in insects, we may divide insects into two main groups, according to what part of the genitalia is the most important in gripping the female. The Diptera are representatives of the one group, in which certain parts of the genitalia are developed into one or two pairs of forceps, while the penis, though it may have hooks to hang on to the female, is not as a rule distensible so as to be able to grip the female by pressure exerted on the walls of the vagina. In the Coleoptera, on the other hand, representative of the other group, there are usually no effective forceps, but part of the penis can be everted, and grips the vagina by being distended within it after insertion. These two groups seem to agree fairly well with divisions of the insects made on general morphological grounds. It is not yet certain how far it is safe to assume that correlation must be the same in these two divisions; here at any rate the form agreeing with the Diptera will be dealt with first.

In fleas it has long been known that the female is seated on the back of the male in copulation; correlation is therefore almost certainly inverse. In the Mecaptera, for which group the evidence is summarised by Stitz (1926), the FVP. is usual, sometimes modified as in *Panorpa*, where the male sits to one side of the female, but seizes her abdomen from beneath. In the Trichoptera the observations are scanty, but Wesenburg-Lund (1913) describes the pairing of *Mystacides nigra* L.; the pose is MVP., but the position is false LP., since the male twists himself round. In the Hymenoptera the usual pose is the MVP. This has been observed in many

bees, wasps and ants. In sawflies, Boulangé has discussed the whole question at great length. Here, in generalised forms such as the Siricidae, the MVP. is adopted; in a large number of forms, however, constituting the division "Strophandria," the hypopygium has been inverted, and in these, as far as the records go, mating is always in the LP.

Finally, the Lepidoptera must be considered, and here unfortunately facts seem to be contradictory. Michael (1923) has instantaneously killed pairs in copula of the silkworm moth (*Bombyx mori* L.), and his dissections show that the linear pose is adopted; the anus is dorsal in all Lepidoptera, so there can be no question of an inverted hypopygium. My own observations on various moths, *e.g. Adela fibulella* F., *Micropteryx* spp., and butterflies, *e.g. Hesperia malvae* L., *Thanaos tages* L., also show the LP., in a modified form. In these species the male approaches the female from behind and copulates by bending his abdomen over his shoulder, so that his genitalia come to grip the female's abdomen just in front of his face. The position is the normal linear one. The curious contortion just described is probably made in the effort of the male to keep his eyes and antennae directed the whole time towards the female he is chasing.

It is very difficult to reconcile this linear pose with the inverse correlation established for all the preceding groups; it is unlikely that the penis is twisted within the vagina, for, if this could happen easily, there would seem to be no reason why the hypopygium should be so often inverted in forms which adopt the LP. There seem to be three possible explanations; first that the Lepidoptera are not closely related to the preceding groups, which is incredible. Secondly, that the penis of the Lepidoptera has been inverted without affecting the rest of the genitalia; Chapman (1902) notes that the actual orifice of the penis is often asymmetrically placed, and suggests that it may be so more often than is generally supposed. If such torsion has occurred it might easily pass undetected unless a very careful study was made of the nerve supply and musculature. Thirdly, it is possible that correlation is not so important and constant as has been suggested; this can only be decided by future observations. Finally, Seitz (1913) describes the normal MVP. for the butterfly, *Anthocharis charlonia* Dup.; evidently the Lepidoptera require further investigation[1].

It is interesting to find that marked asymmetry of the genitalia has been acquired in certain Lepidoptera, notably in the Sphingidae, in the genera allied to *Hemaris* (Chapman, 1902). There are other instances given by Poljanec (1900), Pierce (1909) and Skinner and Williams (1922). In the Hemarines the anus is dorsal, so if there has been any inversion, for which, as far as I know, both positive and negative evidence are lacking, the twist must have been through 360°. The pairing of *Hemaris bombyliformis* Ochs. has been described (anonymous, 1920); unlike most Lepidoptera, copulation takes place on the wing; the pose is not stated, but it may be presumed, from the absence of comment, to be MVP. or FVP. Doubtless further observations on the mating of these forms will throw some light on their asymmetry.

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[1] It may be important that the female genital orifice of most Lepidoptera is a secondarily developed structure.

Since Darwin's observations (1894) there have been many additional records of the respective activities of the sexes of butterflies, when flying paired[1]. Darwin thought that females which were brighter coloured than their males were also more active in courtship, and, in particular, carried the male when the pair were on the wing. It is now proved, however, that his idea was erroneous; in any genus either the female or the male may do the flying (in a few genera either sex), and the differences between genera in this respect, though very diagnostic (Warren, 1920), seem to be unconnected with colour.

The peculiar pairing attitude of dragonflies has already been described (p. 307); it further emphasises the isolation of the order. The mayflies, also, another isolated group, have peculiar habits; usually the male is beneath the female, as in *Ephemera* for instance, his much elongated front legs being extended upwards to grip the female thorax. Bernhard (1907) illustrates the pairing of *Chloeon*; according to his figure the female is on top, but the male has his ventral surface apposed to hers. In the absence of any evidence of inversion of the hypopygium in this genus, and because the front legs in this genus are, as usual, much elongated in the male, I think Bernhard's observation requires confirmation.

In the Neuroptera it appears, from the observations of Withycombe (1922 *a*) and Tillyard (1918), that the linear pose or some modification of it is adopted. In *Sialis fuliginosa* Pict. I have observed a pose exactly like that already mentioned for *Adela*, etc., viz. a modified LP. Smith (1922) also records a modified LP. and a linear position for the Chrysopidae.

Schoenemund (1924) describes the pairing of the Plecoptera, which he states is uniform throughout the group. The male sits to one side of the female, so that the pair are really in the FVP. Samal (1923) gives a very good description of this pose in *Perla abdominalis* Burm. The only pair I have seen of this order, *Nemoura* sp., was, apparently, in a different position. The male was on the back of the female, and his abdomen was twisted so that at its apex the ventral side was uppermost; it was passed under the female to one side of her abdomen. This complicated pose is really a modification of the linear one as regards correlation. Further observations will probably clear up the apparent discrepancy between my single record and those of Schoenemund and other authors.

In the Orthoptera the FVP., or else the false MVP., are the only poses recorded (Poulton, 1896; Gerhardt, 1913 and 1914). Earwigs (Gerhardt, 1913, p. 512), as already mentioned, adopt the false linear pose, so that in them too the correlation is inverse. In cockroaches the evidence is partly contradictory, but Cornelius (quoted in Miall and Denny, 1886, p. 180) records the FVP. for *Blatta*, while Illingworth, in *Periplaneta*, observed the MVP. and the false linear position.

Finally, the groups more or less evidently allied to the Coleoptera have to be considered. The Coleoptera themselves are particularly puzzling; Balfour-Browne (1910) and Donisthorpe (1900) describe the mating of two Hydrophilidae; in this family the penis has simple structure and is quite symmetrical. In these forms it appears that when the penis is exserted the apical part twists through an angle

[1] For accounts of the flight of butterflies when paired see: 70, 76, 86, 87, 309, 352, 376, 391, 394.

of 180°, so that when the whole penis is bent under the male's body for copulation the apical part of the penis comes into direct correlation with the vagina. In the Scarabaeidae Sharp and Muir (1912) record the whole of the penis is twisted through 180° in the adult; the torsion was discovered by comparing adult structures with those of the pupa. Harnisch's study of *Lina populi* L. (1915), which has already been quoted, appears to show inverse correlation; the apparatus is so asymmetrical, however, that this is really very uncertain. In *Lomechusa strumosa* F. Donisthorpe (1907) records the FVP. Probably, in this group, asymmetry has been partly developed in order to accommodate the penis, which is often excessively large, within the abdomen when retracted. The whole problem in this order requires further study. Similarly the Hemiptera have, as yet, been insufficiently studied from this point of view. The researches of Singh-Pruthi (1925) show considerable asymmetry in many groups of Heteroptera, so the result of the usual MVP. is difficult to guess; in forms such as the aphids, in which the genitalia are symmetrical, the MVP. seems also to be the rule. In the Thysanoptera, Buffa (1907) has figured the pose in *Trichothrips copiosus* Uzel. Apparently the insects are in the false linear pose; the pair are not quite tail to tail, but rather at an obtuse angle, and the tip of the male abdomen is twisted through 180°, or nearly. Solowiow (1924) records a similar pose in the Psocid, *Atropos pulsatoria* L., the insects being tail to tail, but with the ventral surfaces of their abdomens apposed.

Consideration of these lesser-known groups of insects shows merely the sort of observations that are required, and does not allow any conclusions to be drawn.

Some suggestions are now offered on the origin and causes of the asymmetry and of the curiously contorted poses that have been described. It must not be supposed that asymmetry is always due to the same factors; the remarkable genitalia of the Blattidae and Mantidae are highly asymmetrical, but there is nothing at present to connect this with the phenomenon of inversion found in the Diptera and Hymenoptera; other examples, probably of a special nature, are seen in the beetles of the sub-family Chauliognathinae (Champion, 1914). Probably, however, the majority of cases of asymmetry are connected with special mating poses and are rightly considered in conjunction with those insects in which actual torsion is found.

There are two quite distinct problems which require explanation; first, the adoption in different species of poses which necessitate (or are necessitated by) torsion; secondly, the reason for the FVP. in some species and the MVP. in others. The second problem which does not involve correlation may be dealt with first. It is almost self-evident that the MVP. is the most natural one, since the female is known by observation to require coercion in many forms. Modifications of the MVP. are thus to be considered secondary. I have already explained that, in the MVP., the female anus is put out of action by the pressure of the male venter. I think that there may be some correlation between a pose which leaves the female anus free, and need of special food in the female, during or just before copulation. The FVP. and the false MVP., the two poses in which the female anus is most completely disengaged in copulation, are found mainly in species in which feeding is intimately connected with mating, or in which, at least, the female is voracious, while the male

eats little or not at all. In the grasshopper, *Podisma* (*Pezotettix*) *pedestre* L., Poulton (1896) noticed that the female sometimes passed faeces of a special character just before copulating. If more evidence for this were obtained in other groups there would be a case for this theory. Once the need for the free female anus is explained, the false MVP. might well become usual in order that the male might grip the female more efficiently. The linear or false linear poses also allow the female anus to be more or less free, but these poses are inconvenient, as a general rule, for active locomotion, and throw all the strain of gripping the female on the genitalia.

The problem of torsion is an even more difficult one, and raises certain points of interest to the general theory of heredity. Only two authors besides Lamb, as far as I know, have attempted explanations. Chapman (1902), dealing with the asymmetry in the hawkmoths (*Hemaris*, etc.), suggests that as many moths copulate when seated side by side, and unequal strain is thrown on the claspers, a greater development of those of the side which is furthest from the female might be expected. This does not of course explain why a pose involving unequal strains should be adopted; it might be suggested that the lateral position was necessary because the scales of Lepidoptera offered difficulties to a secure MVP. This is, however, a rather uncertain assumption, and does not help in the other groups. Probably all the variations are due to a common cause. It is not possible to prove whether asymmetry conditions a special pose or vice versa; but the false linear pose found in species not very distantly allied to forms with inverted hypopygia, strongly suggests that asymmetry is preceded by a pose which makes a twist of the abdomen necessary if the original correlation is to be preserved. The earwigs (Dermaptera) provide evidence of special value on this point; it is almost certain that the callipers of typical earwigs make a FVP. or MVP. impossible, so that to maintain the indirect correlation the false linear pose is adopted. Now the Orthoptera all adopt the FVP., and these insects (as also such peculiar earwigs as *Arixenia*) do not have apical abdominal appendages so developed as to make this pose difficult. It seems probable that the earwigs must first have adopted the false linear pose, and afterwards "grew" the callipers which that pose allowed. It is unlikely that they would first develop structures that made copulation in the FVP. difficult, and be so driven to the false LP.

In the sawflies, Boulangé (1924) hints that the MVP. offers difficulties to some of the species which have a short stout abdomen in the male. I suppose that Boulangé would regard the false linear pose as easier for such forms than the MVP., and would suppose that the twist of the abdomen demanded by the former pose is sooner or later obviated by the inversion of the hypopygium. I think we must admit at present that there is no adequate explanation of the change from the MVP. to the false linear; there is evidently some good reason for it, and a reason which is applicable to all insects; there is no other way of accounting for the frequent occurrence of inversion in unrelated groups, and for the fact that, even in not very distant families such as the Culicidae and Psychodidae (Feuerborn, 1922 *a*), the torsion takes place between different segments. Further, I find that the twist has been from left to right in *Bombylius discolor* Mikan, but from right to left in *Volucella pellucens* L., and *Calliphora*. It is still more difficult to account for the circumversion of the

hypopygium in the Muscidae, etc. (including the majority probably of the species of flies). There is, of course, very little evidence that these forms have passed through a stage of simple inversion, but in our present ignorance it is natural to suppose that this has been the case.

Finally, we may consider the mechanism by which the twist is brought about in the various groups. In the sawflies, Boulangé shows that the shape of the hypopygium is such that its normal position is one of unstable equilibrium, so that movements of neighbouring muscles will sooner or later result in inversion, and, consequently, a stable position. This is evidently a specialised condition, since the hypopygium has had its shape altered so as to make inversion easy. In the less modified forms such as the Tipulids, the mosquitoes and Psychodids, there is no asymmetry of the skeleton, but soon after emergence certain intersegmental muscles contract permanently, so as to make a twist in one of the arthrodial membranes. It is a remarkable fact that the twisting should not take place till after emergence, and this is probably only the case when there is no marked skeletal asymmetry. In *Bombylius discolor* Mikan there is a certain degree of asymmetry in the segment preceding the hypopygium, and very likely in this form twisting takes place earlier. In sawflies twisting takes place just before emergence (Boulangé, 1924). In *Calliphora* (my own observations) the torsion occurs much earlier, apparently at a very early pupal stage. In this genus, too, there is marked skeletal asymmetry. In *Hemaris* (Poulton, 1890) the genital rudiments of the pupa are asymmetrically placed. These facts undoubtedly suggest, though they by no means prove, that the twist exerted on the abdomen by the false L.P. has eventually led to hereditary torsion resulting finally in skeletal asymmetry. It is equally conceivable that, if torsion was necessary, variations in the right direction might have been preserved, had they happened to occur. The choice between these suggestions will be guided by the theory of heredity supported by the reader. The special difficulty of the first "explanation" is that there is no evidence that the abdominal twist, as distinct from actual inversion, always takes place in the same direction in any one species. (Cf. Riesen, 1909.)

It has often been pointed out that secondary sexual characters (like the torsion just mentioned) as a whole tend to develop late in the life of the insect (*e.g.* Cunningham, 1900). In such forms as the mayflies, for instance, the long front legs of the male do not differ from those of the female till after the last moult; in this group there is a winged stage, the subimago, before the adult, and the secondary sexual characters appear only after the change into the final form, not in the subimago (Ulmer, 1924). Similarly, the sexual dimorphism in colour, obtaining in various dragonflies, does not develop till some time after emergence (Williamson, 1905), nor, in the grasshoppers, does the stridulatory apparatus, as a rule, develop till the last moult (Petrunkewitsch and von Guaita, 1901). In some Orthoptera, on the other hand, *e.g. Gongylus* (Williams, 1904), where development is more gradual than in dragonflies or mayflies, the male characters are in part acquired early and slowly become more marked with growth. The significance of such facts in insects is at present doubtful.

16. FINAL STATEMENT WITH REGARD TO FEMALE COYNESS.

All the factors I have been able to discover bearing on the coyness of the female may now be summarised. It can be established, in the first place, that in some species coyness is only exhibited when copulation would be ineffective (*e.g.* when the spermatheca is already full), while in others there is no coyness at all. In some moths or beetles (cf. Balfour-Browne, 1910) the female, when ready for copulation, will accept, apparently, any male, while if she is in a physiologically unsuitable condition, no male will persuade her (cf. the behaviour of *Neurigona*; see p. 308). In short-lived forms (*e.g. Orgyia*, Freiling, 1909) the female may be eager for copulation the moment she has emerged. In fact, in some species (*Gynaephora*, Johannsen, 1921; *Scleroderma*, Wheeler, 1924 *a*; *Heliconius*, Hering, 1926, p. 144; *Opifex*, Kirk, 1923) the males copulate with the female at the instant of emergence, often gathering round the pupa, and sometimes cutting their way into it. In the mayflies of the genera *Palingenia* and *Campsurus* (Ulmer, 1924) as a general rule, and in *Ephemera danica*, Müll. (Venour, 1906), as an exception, the male may pair with the subimago stage of the female; in the first two genera the female of several species is unknown in the adult stage.

More often some delay is necessary before pairing can take place; although the gonads and the associated glands may mature in this interval, there is seldom any connection proved at present between immaturity of the gonads and unwillingness to pair or effectiveness of fertilisation (cf. Petersen, 1892). In this connection Knab's observation that certain Chrysomelid beetles live through the winter before they acquire either sexual instincts or the bright, often metallic, adult colours, may be mentioned.

The existence of elaborate displays in the males of many species, and the frequent independent evolution of complex scent-organs, whose only function seems to be to excite the female, lead irresistibly to the conclusion that there must be a further type of reluctance (besides mere immaturity), which it is advantageous for the male to overcome as quickly as he can. In some forms it may be that the need of special food makes the female coy until this has been provided; in some cases the male seems to overcome this difficulty by offering either his captured booty or himself, to satisfy the female. In other cases the structure of the genitalia apparently makes peculiar attitudes necessary in copulation. This may have led to the acquisition of displays or odours (or food-production in *Panorpa*) which are so attractive to the female that she is induced to remain motionless while the male makes whatever contortions are necessary. The Empididae are especially interesting in showing how the behaviour of the male may be due to various causes. Here we may suppose that the prey he provides acts partly, probably, as a lure to the female, partly also is necessary to the development of the eggs, and partly, by the action of the two former processes on the female, allows pairing in the FVP. to take place in the air.

The life of an adult insect may be divided into a number of activities, such as eating, sleeping, mating or ovipositing, each, perhaps, characterised by a special

psychic state. The passage from one state to another is normally the result of the exhaustion of the effects of one stimulus and the reception of another. Prof. J. S. Huxley has suggested to me that the male's display may be directed not so much towards overcoming a coyness in the female, as to stimulate her to pass as quickly as possible into her "mating-state" from some other normal activity, in which she might have continued for some time. This suggestion that the female is not coy but merely otherwise engaged, might apply to a few insects, but, in many forms, the virgin females seem to have no occupation but waiting for the males, whose advances, nevertheless, they reject at first.

Again, it is possible, as has already been suggested, that coyness may, in some forms, be beneficial by hindering over-copulation, though it is very improbable that this can be the general explanation.

It may be objected that coyness in the female is part of the fundamental sexual dimorphism. This, of course, is possible, but in that case the following facts require explanation: (1) That elaborate displays have so often been evolved, rather than a gradual reduction in the dimorphism in coyness. (2) That the females of many species lack coyness, often copulating before or immediately after emergence. (3) The reversal of the normal sexual rôles in such forms as *Scellus* (p. 309), *Osmylus* (p. 321), *Hepialus* and *Biston* (p. 320), and various Empididae (p. 309). This last fact, indeed, is a difficulty in any explanation.

Evidently there is still much obscurity surrounding the problem of female coyness, especially its physiology.

17. DEFINITE EVIDENCE OF SELECTION.

There are few species in which it has been proved or suggested that a definite preference is shown amongst variations within the species. The term preference is used in its widest sense to include assortative mating due to mechanical necessities. Most of the evidence is suggestive rather than conclusive. The individuals of a species may show differences in size or colour; in a few species experiments have been made in painting out the natural colour-pattern.

Many insects, especially, for instance, beetles feeding in wood or *Polyporus*, vary remarkably in size. Thus, Peringuey (1884), in the South African Longicorn beetle *Cacosceles oedipus* Newm., records that the length in the male varies from 35–54 mm., and in the female from 48–57 mm. In the beetle *Osphya bipunctata* F., Edwards (1907) found great variation in size; in copulation the male seizes the neck of the female with his mandibles, while his modified hind legs grip her venter. It was found, in confinement, that large females always escaped from small males, who were unable to hold them, while the small females easily slipped away from beneath the large males. Similarly, Grosvenor (1921) found that a large race of the moth *Zygaena trifolii* Esp. from Sussex and a small one from Kent were unable to interbreed, though eager to do so. The interest of this lies especially in the fact that the species of a genus can often be divided into groups according to size. In a species where this kind of assortative mating was usual, a heritable size-variation might eventually split up the species into two non-interbreeding forms. Probably

a little observation would show that size often had a selective effect in mating. There are species however—*e.g. Xylotrupes gideon* L. (Bateson and Brindley, 1892)— in which the small and large individuals often mate.

A rather different case is described by Poisson (1924) in the water-strider, *Limnotrechus (Gerris) lacustris* L. Here, there is considerable variation in wing-development, with which certain features in the thorax and genital segments are also correlated. According to Poisson's observations (based, it is true, on rather small numbers) the macropterous and brachypterous forms, when they occur together, only interbreed to a limited extent. His breeding experiments also suggest that the so-called species *L. costae* H-S. and *L. thoracica* Sch. are really dimorphic forms of one species, which seldom or never interbreed. On one occasion he bred one form out of the other. These results obviously require confirmation, but they are already very suggestive. This type of wing-dimorphism is common in the Hemiptera, and is sometimes a specific character.

Grosvenor (1921) obtained some evidence that a black variety of *Zygaena trifolii* Esp. would only pair with other specimens of the variety with great reluctance, while a black female was apparently more attractive to a normal male than was a normal female. An albino female was entirely without attraction to normal males. Carpenter (1913 a) noted that in the very variable moth *Epitoxis albicincta* Hampsn. there seemed to be a tendency for like varieties to mate with like. On the other hand, many polymorphic species are known (*e.g. Donacia* spp. *Volucella* and other Syrphids; *Papilio dardanus*, Brown etc.) in which all the forms interbreed, but there is usually no evidence available to show whether any of the types of cross-pairings are commoner than others. In the variable ladybird, *Hippodamia convergens* Guér., however, Kellogg (1906) shows that matings certainly take place quite without reference to colour.

Mayer (1900) showed that colour plays no part in the mating of *Callosamia promethea* Drury (see this paper, p. 300). In *Lymantria (Porthetria) dispar* L., the gypsy moth, Mayer and Soule (1906) found that mating was unaffected by painting the male's wings bright green or scarlet. On the other hand, males whose wings had been removed were not so successful in mating as normal males; this was probably due to the visual selection of the female, for, if her eyes were plastered over with varnish, this preference was not shown. Observations of Eltringham and Seitz (see p. 314), however, that colour may be important in some butterflies.

Crampton (1904) studied the matings of moths whose pupae he had examined statistically and found that, on the whole, insects derived from pupae approximating to a modal type were most successful in mating; individuals emerging from pupae aberrant from that type formed the majority of the insects which failed to mate. This selection results in keeping the strain pure; it was not shown that similar variations from the mean would mate as a rule with one another.

Those who have been successful in crossing species rarely seem to give any account of the behaviour of the insects in mating. Soule (1902) succeeded in crossing *Philosamia (Samia) cynthia* Grote with *Callosamia (Attacus) promethea* by putting a male and female of these species together in one compartment, and making a

current of air pass in from another compartment containing the true female of the male's species. In these Saturniids, of course, mating is almost entirely controlled by the scent of the female. In the Bistonines, on the other hand, Harrison (1913) found this method useless; when once the atmospheric conditions were right, pairing often took place at once, though there was much, apparently capricious, variation in the readiness of different species to pair. Seitz (1894) suggests that moths have two scents, a specific and a sexual one, so that if the male and female of different species are closely confined, the latter scent may overcome the former.

There seems to be little evidence that hybrids are common in nature, even in species which are easily crossed in confinement. Probably the combination of slight differences in habitat, time of appearance, and mating reactions (scents, etc.), when taken together, might stop interbreeding almost completely. The tendency for like to mate with like within the species would obviously be even more effective in restricting specific crossing. If the first barrier in the divergence of species were a physiological one of this nature, it would be easy to see why the sterility of interspecific crosses should be so capricious in its incidence, for it might arise, more or less accidentally, any time after the species had ceased to interbreed.

18. SUMMARY AND CONCLUSIONS.

(1) In the majority of insects there are differences of structure or behaviour between the sexes; these differences are additional to the primary dimorphism in chromosome constitution and in the nature of the gonads and their ducts.

(2) If the dimorphism due merely to the special maternal functions of the female be excluded, there remain differences of two main types, viz. (*a*) non-epigamic, and (*b*) epigamic.

(3) The non-epigamic characters are either secondary results of the primary dimorphism (*e.g.* colour in *Lymantria*, Goldschmidt, 1923) or are due to a dimorphism in the laws of growth (*e.g.* the horns of the Scarabaeidae); the exact way in which such growth is controlled, and what function, if any, the resulting structures fulfil in the life of the animal concerned, are not at present understood. Dimorphism in the laws of growth cannot logically be separated from the primary dimorphism, except that it appears improbable that such elaborate end-results should be reached merely accidentally.

(4) Epigamic characters may be divided into (*a*) those which are practically essential to one step or another in the series of reactions which lead up to successful mating; (*b*) those which reduce the time intervening between the meeting of the sexes and successful copulation; and (*c*) those which give a male (or, in special cases, a female) advantages over rivals of the same sex.

(5) Characters of the type (4 *a*) include scent-organs, light- or sound-producing organs in the female (and in the male, too, in the case of light- and in some sound-producing organs); highly developed sense-organs in the male; clasping-organs in the males: perhaps also, in some forms, bright colours (whether dimorphic or not) which serve as recognition marks. In insects, such characters are often marvellously developed; they present the special problems of extreme specific diversity.

It is impossible, as a rule, to say why an organ apparently very useful in mating (*e.g.* modified front tarsi) should be so much more developed in some species than in others, and should have such a diversity of form.

(6) Characters of the type (4 *b*) include scent-organs, and sound-producing apparatus in the male (in most cases; scent-organs of *Osmylus*, *Hepialus*, etc. are comparable to those of females); most of the behaviour classed as display, including special movements of the wings or legs, special modes of flight, stroking of the female, etc.; in certain cases, gifts of food to the female. Such characters are extraordinarily developed in many insects, often modifying the whole body (*e.g.* the grasshopper *Pneumora*; male Psychodidae). Since males have so often acquired special structures or behaviour merely in order to excite the female, it must be supposed that the female is not at first willing to mate (even when she is in a condition in which copulation would be effective), and further that the male display overcomes this "coyness."

(7) We conclude, then, that the female is coy even at times when copulation would be successful, for no purpose could be served by the male attempting to overcome a coyness due to physiological necessities. This coyness requires explanation, because it seems, at first sight, dangerous to the species by delaying copulation. Conversely, this danger admitted, the displays, and the structures employed in them, have survival value. Although the displays, considered as a whole, have a survival value, yet it is difficult to see any use in the specific differences in secondary sexual structures of behaviour. It is not at all evident how selection could establish, in a population, a character whose only effect would be to isolate individuals possessing it from those who did not. In other words, though it is advantageous to shorten the interval between the meeting of the sexes and the end of an effective copulation, there seems no advantage in procuring this result in so many different ways.

(8) In some insects there are possible reasons for real or apparent coyness, viz. (1) the coyness may tend to preserve the species from the danger of over-copulation, especially when, as often happens, the male is, in effect, much the more numerous sex. (2) Some male behaviour may be directed not so much to exciting the female, as to providing her with necessary food (*Panorpa*; *Oecanthus*; Empididae). (3) The apparent necessity for the orientation of the male intromittent organ within the female vagina to remain constant throughout large groups of insects has led to the adoption of various strange attitudes in copulation; the male's display may sometimes facilitate copulation by immobilising the female while he places himself in the necessary position.

(9) The basis of coyness remains, however, undiscovered in the majority of cases, and very little is known of the actual physiological differences between the sexes which lead to one sex being eager and the other reluctant. It might be assumed that female coyness was part of the primary dimorphism; but this is unlikely, for in species which have special reasons for mating as quickly as possible (*e.g.* short-lived moths) the female may signal for the other sex by scent-emission from the moment of emergence, and copulation may even be effected while the female is still

in the pupa (cf. Alexander, 1920). Further, in some species the female is as active as the male (some Empididae) or even more so (*Hepialus hectus* L. and *humuli* L.; *Ctenophora, Scellus virago* Ald.; *Osmylus*). Coyness, therefore, remains in the main unexplained, especially in its physiological aspect.

(10) Characters of the type (4 *c*) include organs specially developed for fighting (*e.g.* perhaps the mandibles of the Lucanidae; the elytral spines of *Platypus*). Other characters, such as greater strength or powers of flight or vision, may perhaps also be included here; such powers, however, would be influenced by so many factors that it is impossible to say to what extent competition with other males alone has been effective. Most of the structures discussed in (6) were formerly included here. This was probably incorrect because there is often little evidence that the male display is at all competitive, and because it is probable in many species (though only proved in *Drosophila*) that the female, when once roused, will mate with any male.

(11) The little positive evidence that exists seems to show that assortative mating within the species usually leads to like mating with like, to the disadvantage of variations, in either direction, from the mean. This naturally tends to stop species from intercrossing. Probably in insects the main barriers to interspecific crossing are essentially physiological (*e.g.* differences in time of appearance, in habitat, in the scents produced, etc.); differences in genitalia may occasionally also be effective (cf. Harnisch, 1915), but as a rule species differing in genitalia do not commonly attempt to pair in nature. How these differences arise, and how each sex comes to be altered in the appropriate way more or less simultaneously, is practically unknown.

(12) Throughout the present paper it has been assumed that complex structures or elaborate displays in the male must be useful either to him or to the species. It could, however, be supposed in some cases that the structure or the display has no special survival value, and was comparable to the other, apparently useless, characters found in many animals. There is no space here to argue the question of the powers of natural selection in general.

19. BIBLIOGRAPHY.

The bibliography of the present paper must be regarded as merely an introduction to the vast literature of the subject. In choosing papers for the following list certain principles have been adopted. It is always found that there are dozens of descriptions of the anatomy of an organ for one good account of its function in nature. I have, therefore, included as a rule only a few of the anatomical researches published on any subject, but have put in everything I could find dealing with the use of structures in courtship. It is unlikely that, even in the latter respect, the list is complete, especially since papers with much relevant matter often have misleading titles. Much further bibliographical information will be found in many of the papers mentioned, and especially in Meisenheimer (1921).

Besides being arranged alphabetically, according to authors, the papers are numbered consecutively, in order that short bibliographies, referring to papers by the number only, may be given in footnotes to the text.

(1) ADLER, H. (1894). *Alternating Generations; a Study of Oak-galls and Gall-flies.* Translated by C. R. STRATTON. Oxford.

(2) ADLERZ, G. (1912). "Lefnadsförhållanden och instinkter inom familjerna Pompilidae och Sphegidae." K. *Svenska vet. Akad. Handl.* **4**, no. 10, 1.

(3) AINSLIE, C. N. (1907). "Notes on the swarming of a species of crane-fly." *Canad. Ent.* **39**, 26.

(4) ALDRICH, J. M. (1894). "Courtship among flies." *Amer. Nat.* **28**, 35.

(5) ALDRICH, J. M. and TURLEY, L. A. (1899). "A balloon-making fly." *Amer. Nat.* **33**, 809.

(6) ALDRICH, J. M. (1906). "The Dipterous genus *Calotarsa*, with one new species." *Ent. News*, **17**, 123.

(7) ALEXANDER, C. P. (1920). "The crane flies of New York. Part 2, Biology and Phylogeny." *Cornell Univ. Agric. Exper. Sta. Mem.* **3** (p. 705, mating of *Eriocera longicornis* (Walk.); pp. 710–12, mating-habits of the family).

(8) ALLARD, H. A. (1916). "The synchronal flashing of fireflies." *Science*, N.S. **44**, 710.

(9) —— (1917). "Synchronism and synchronic rhythm in the behaviour of certain creatures." *Amer. Nat.* **51**, 438.

(10) —— (1918). "Rhythmic synchronism in the chirping of certain crickets and locusts." *Amer. Nat.* **52**, 548.

(11) —— (1920). "The flight of fireflies and the flashing impulse." *Science*, N.S. **52**, 539.

(12) ALSTON, A. M. (1920). "The life-histories and habits of two parasites of blowflies." *Proc. Zool. Soc.* p. 195.

(13) ANONYMOUS (1920). No title. (Pairing of *Hemaris bombyliformis* Och.) From Rep. of entom. section of Somersetshire Arch. and Nat. Hist. Soc.; in the *Transactions* of the Society, **46**, p. lxiii.

(14) ANTRAM, C. B. (1908). "Sexual attraction in Lepidoptera." *Journ. Bombay Nat. Hist. Soc.* **18**, 923.

(15) ARCHER, H. (1884). "Bigamy in *Platipteryx hamula*." *Ent. Mo. Mag.* **20**, 228.

(16) ARROW, G. J. (1920). "Horned beetles." *Proc. Ent. Soc. Lond.* 3 March, p. xviii.

(17) BALFOUR-BROWNE, F. (1910). "On the life-history of *Hydrobius fuscipes*." *Trans. Roy. Soc. Edinburgh*, **47**, 317.

(18) —— (1922). "On the life-history of *Melittobia acasta* Walker; a chalcid parasite of bees and wasps." *Parasitology*, **14**, 349.

(19) BALL, F. J. (1914). "Le dimorphisme saisonnal des androconia chez certaines Rhopalocères." *Ann. Soc. ent. Belge*, **58**, 170.

(20) BARLOW, J. (1921). "The mating habits of *Megarhyssa* (Hym., Ichneumonidae)." *Ent. News*, **32**, 291.

(21) BARNES, H. F. (1924). "Some observations on the mating habits and oviposition of the Limnobiidae (Diptera)." *Ent. Mo. Mag.* **60**, 71.

(22) BARNES, P. T. (1919). "Fireflies flashing in unison." *Science*, N.S. **49**, 72.

(23) BARRETT, C. G. (1882). "Odour emitted by the male of *Hepialus hectus*." *Ent. Mo. Mag.* **19**, 90.

(24) —— (1885). "Curious performance of a Noctua." *Ent. Mo. Mag.* **22**, 112.

(25) —— (1886). "Singular habit of *Hepialus hectus*." *Ent. Mo. Mag.* **23**, 110.

(26) BATESON, W. and BRINDLEY, H. H. (1892). "On some cases of variation in secondary sexual characters, statistically examined." *Proc. Zool. Soc.* p. 585.

(27) BEQUAERT, J. (1919). "A revision of the Vespidae of the Belgian Congo, etc." *Bull. Amer. Mus. Nat. Hist.* **39**, 1.

(28) BERNHARD, C. (1907). "Ueber die vivipare Ephemeride, *Chloeon dipterum*." *Biol. Centr.* **27**, 467.

(29) BERTKAU, P. (1882). "Duftapparat von *Hepialus hectus*." *Arch. f. Naturges.* **48**, 363.

(30) —— (1887). "Scent organs of German Lepidoptera." Abstract in *Journ. Microscop. Soc.* (1888) **1**, 406.

(31) BETHUNE-BAKER, B. T. (1922). "Monograph of the genus *Catachrysops* Boisd. (auctt.)." *Trans. Ent. Soc. Lond.* p. 275.

(32) —— (1925). "On the scent-sacs in the genus *Rhodogastria*." *Trans. Ent. Soc. Lond.* p. 321.

(33) BEZZI, M. (1892). "Contribuzione alla Fauna Ditterologica della Provinzia di Pavia." *Boll. Soc. Entom. Ital.* **26**, 97 (see p. 115).

(34) BLAIR, K. G. (1915). "Luminous insects." *Nature*, **96**, 411.

(35) —— (1924). "Some notes on the luminosity of insects." *Ent. Mo. Mag.* **60**, 173.

(36) —— (1926). "On the luminosity of *Pyrophorus* (Coleoptera)." *Ent. Mo. Mag.* **62**, 11.

(37) BLUNCK, H. (1912). "Das Geschlechtsleben des *Dytiscus marginalis* L." *Zeitschr. f. Wiss. Zool.* **102**, 169.

(38) —— (1913). "Beitraege zur Naturgeschichte des *Dytiscus marginalis* L." *Zool. Jahrb. Syst.* **30**, 1.

(39) —— (1924). "Lebensdauer, Fortpflanzungvermoegen und Alterscheinungen beim Gellbrand (*Dytiscus marginalis* L.)." *Zool. Anz.* **58**, 163.

(40) BOLDYREV, B. T. (1912). "Liebeswerben und Spermatophoren bei einigen Locustodeen und Grylliden." *Hor. Ent. Soc. Rossicae*, **40**, 1.
(41) BOULANGÉ, H. (1924). "Recherches sur l'appareil copulateur des Hyménoptères et spéciale-ment des Chalastogastres." *Mém. et Trav. des Facultés Catholiques de Lille*, Fasc. xxviii.
(42) BRISTOWE, W. S. (1924). "Notes on the habits of insects and spiders in Brazil." *Trans. Ent. Soc. Lond.* p. 487.
(43) BROWN, R. (1918). "Courtship of *Pararge megaera*." *Entomologist*, **51**, 233.
(44) BRUNETTI, E. (1912). *Fauna of British India. Diptera Nematocera.* London.
(45) BRYK, F. (1919). "Bibliotheca Sphragidologica." *Arch. f. Naturges* **85**, Heft 5, 102.
(46) BUCKHURST, A. S. (1926). "*Gastrophilus equi* on mountain tops." *Ent. Mo. Mag.* **62**, 60.
(47) BUFFA, P. (1907). "Alcune notizie anatomiche sui Tisanotteri Tubuliferi." *Redia*, **4**, 369.
(48) BUGNION, E. (1916). "Les insectes phosphorescents." *Bull. Murithienne*, **39**, 82.
(49) BUTLER, E. A. (1923). *A Biology of the British Hemiptera-Heteroptera.* London.
(50) CAMBELL, W. HOWARD (1908). "Sexual attraction in Lepidoptera." *Journ. Bombay Nat. Hist. Soc.* **18**, 511.
(51) CAMPION, H. (1915). "The copulation of scorpion-flies." *Entomologist*, **48**, 123.
(52) CARPENTER, G. D. H. (1912). "Progress report on investigations into the bionomics of *Glossina palpalis*, July 27, 1910 to Aug. 5, 1911." *Rep. Sleeping Sickness Commission of Roy. Soc.* **12**, 79.
(53) —— (1913 a). "The importance of preserving insects found in coitu." *Proc. Ent. Soc. Lond.* 5 Nov., p. lxxxiii.
(54) ——(1913 b). "Second report on the bionomics of *Glossina fuscipes* (·· *palpalis*)." *Rep. Sleeping Sickness Commission of Roy. Soc.* **14**, 1.
(55) ——ᵣ (1914). *See* POULTON, E. B. (1914).
(56) —— (1918). *See* POULTON, E. B. (1918 b) and DIXEY, F. A. (1918).
(57) CARTER, A. E. J. "Diptera in Scotland." *Ent. Mo. Mag.* **41**, 163.
(58) CAUDELL, A. N. (1909). "The copulation and feeding habits of *Idiarthron atrispinis* Stål." *Proc. Ent. Soc. Washington*, **11**, 40.
(59) CHAMPION, G. C. (1907) (for EDWARDS, J.). "Forms of *Osphya* and a concurrent species." *Proc. Ent. Soc. Lond.* 10 April, p. xxiv.
(60) —— (1914). "A revision of the Mexican and Central American Chauliognathinae (family Telephoridae), etc." *Trans. Ent. Soc. Lond.* p. 128.
(61) CHAMPLAIN, A. B. (1921). "The curious mating habit of *Megarhyssa atrata* F." *Ent. News*, **32**, 241.
(62) CHAPMAN, T. A. (1869–70). "Note on the pairing of *Odynerus spinipes* L." *Ent. Mo. Mag.* **6**, 214.
(63) —— (1891). "A query as to hibernation." *Ent. Mo. Mag.* **27**, 22.
(64) —— (1902). "On the asymmetry in the male of Hemarine and other Sphinges." *Trans. Ent. Soc. Lond.* p. 679.
(65) ——·· (1916). "The pairing of Plebeid blue butterflies." *Trans. Ent. Soc. Lond.* p. 151; correc-tion, p. 297.
(66) CHRISTOPHERS, S. K. (1915). "The male genitalia of *Anopheles*." *Indian Journ. Med. Research*, **3**, 37.
(67) COBLENTZ, W. W. (1911). "The color of the light emitted by Lampyridae." *Canad. Ent.* **43**, 355.
(68) COCHRANE, A. M. (1909). "Observations on a sexual habit of *Leptidia sinapis*." *Ent. Record*, **21**, 201.
(69) COCKLE, J. W. (1908). "The mating of *Boreus californicus*." *Canad. Ent.* **40**, 101.
(70) COLTHRUP, C. W. (1919). "Paired Lepidoptera in flight, 1918." *Ent. Record*, **31**, 75.
(71) CRAMPTON, H. E. (1903–4). "Variation and selection in Saturniid Lepidoptera." *Biol. Bull.* **6**, 310.
(72) CUMMINGS, B. F. (1914). "Scent-organs in the Trichopteron *Sericostoma personatum*." *Proc. Zool. Soc.* **1**, 459.
(73) CUNNINGHAM, J. C. (1900). *Sexual Dimorphism in the Animal Kingdom.* London.
(74) CUTHBERTSON, A. (1926). "Studies in Clyde Craneflies; the swarming of Craneflies." *Ent. Mo. Mag.* **62**, 36.
(75) DAHL, F. (1889). "Die Insekten koennen Formen unterscheiden." *Zool. Anz.* **12**, 243.
(76) DARWIN, C. (1894). *The Descent of Man and Selection in Relation to Sex.* 2nd ed. London.
(77) DEGEENER, P. (1902). "Duftorgan von *Hepialus hectus*." *Zeitschr. f. Wiss. Zool.* **71**, 276.
(78) DEGEENER, P. and SCHAPOSCHNIKOW, C. (1905). "Das Duftorgan von *Phassus schamyl*." *Zeitschr. f. Wiss. Zool.* **78**, 245.
(79) DELL, J. A. (1905). "The structure and life-history of *Psychoda sexpunctata* Curt." *Trans. Ent. Soc. Lond.* p. 293.
(80) DELMAS, R. (1926). "Notes sur la biologie de '*Pristiphora conjugata*' Dahlbom." *Bull. Biol. France et Belgique*, **50**, 447.

(81) DEMOLL, R. (1908). "Die Bedeutung der Proterandrie bei Insekten." *Zool. Jahrb. Syst.* **26**, 621.

(82) DEWITZ, H. (1884–5). "Maennlicher Geschlechtscharakter bei *Catocala*." *Biol. Centralbl.* **4**, 724.

(83) DIXEY, F. A. (1905). No title. (Use of scent in the courtship of Pierines.) *Proc. Ent. Soc. Lond.* 1 Nov. p. liv.

(84) —— (1906). No title. (Scents of South African butterflies.) *Proc. Ent. Soc. Lond.* 7 Feb. p. ii.

(85) —— (1910). "Scents of butterflies." *Proc. Ent. Soc. Lond.* 4 Oct. p. lxxii.

(86) —— (1917). "Nuptial flight of butterflies." *Proc. Ent. Soc. Lond.* 7 Nov. p. ii.

(87) —— (1918). "Capt. G. D. H. Carpenter's Pierines from Lulangru and St Michael's mission." *Proc. Ent. Soc. Lond.* 5 June, p. cl.

(88) —— (1920). "Seasonal dimorphism in androconia." *Proc. Ent. Soc. Lond.* 3 March, p. xiii

(89) DOANE, R. W. (1907). "Notes on the habits of *Scellus virago* Ald." *Ent. News*, **18**, 136.

(90) DOENITZ, W. (1887). "Ein singender Schmetterling." *Berlin. Ent. Zeitschr.* **31**, 9.

(91) DONISTHORPE, H. ST J. K. (1900). "Notes on the copulation of *Hydrophilus piceus* L." *Ent. Record*, **12**, 291.

(92) —— (1907). "The life-history and occurrence of *Lomechusa strumosa*." *Trans. Ent. Soc. Lond.* p. 410.

(93) —— (1917). "Attitudes of Wasps and Psocids in copulation." *Ent. Record*, **29**, 231.

(94) DRENKELFORT, H. (1910). "Biologie und Anatomie von *Siphlurus lacustris* Eaton." *Zool. Jahrb. Anat.* **29**, 527.

(95) DUDICH, E. (1923). "Ueber die Variation des *Cyclommatus tarandus* Thunberg (Coleopt., Lucanidae)." *Arch. f. Naturges* **89**, Heft 2, 62.

(96) DUMONT, C. (1920). "Note biologique sur *Rhytirrhinus surcoufi* Peyerh." *Bull. Soc. Ent. France*, p. 119.

(97) EATON, A. E. (1883–8). "A revisional monograph of recent Ephemeridae or mayflies." *Trans. Linn. Soc. Zool. Lond.* p. 1.

(98) —— (1901). No title. (Eversible glands of male Psychodids.) *Proc. Ent. Soc. Lond.* 6 Dec. p. xxvii.

(99) ECKSTEIN, K. (1892). "Der Baumweissling, *Aporia crataegi* Hb." *Zool. Jahrb. Syst.* **6**, 230.

(100) —— (1911). "Beitraege zur Kenntnis der Kiefernspinner *Lasiocampa* (*Gastropacha*, *Dendrolimus*) *pini* L." *Zool. Jahrb. Syst.* **31**, 59.

(101) EDITORS (1925). "Chironomid flies cause an alarm of fire." *Ent. Mo. Mag.* **61**, 23.

(102) EDWARDS, F. W. (1920 a). "The nomenclature of the parts of the male hypopygium of Diptera Nematocera, with special reference to mosquitoes." *Ann. Trop. Med. and Parasitol.* **14**, 23.

(103) —— (1920 b). "Some records of predaceous Ceratopogoninae (Diptera)." *Ent. Mo. Mag.* **56**, 203.

(104) —— (1920 c). "Scent-organs (?) in female midges of the *Palpomyia* group." *Ann. Mag. Nat. Hist.* (9), **6**, 365.

(105) —— (1923). "New and old observations on Ceratopogonine midges attacking other insects." *Ann. Trop. Med. and Parasitol.* **17**, 19.

(106) —— (1924). "New data concerning *Styringomyia* (Diptera, Tipulidae)." *Ann. Mag. Nat. Hist.* **13** (9 ser.), p. 265.

(107) —— (1926 a). "Extraordinary mating habits of a mosquito." (Quoting Kirk, 1923.) *Ent. Mo. Mag.* **62**, 23.

(108) —— (1926 b). "On the British biting midges (Diptera, Ceratopogonidae)." *Trans. Ent. Soc. Lond.* p. 389.

EDWARDS, J. (1907). *See* CHAMPION, G. C. (1907).

(109) EGGERS, F. (1919). "Das thoracale bitympanale Organ einer Gruppe der Lepidopteren Heteroceren." *Zool. Jahrb. Abt. f. Anat.* **41**, 273.

(110) ELTRINGHAM, H. (1910). *African Mimetic Butterflies.* Oxford.

(111) —— (1913). "On the scent apparatus of the male *Amauris niavius* Linn." *Trans. Ent. Soc. Lond.* p. 399.

(112) —— (1915). "Further observations on the structure of the scent-organs of certain male Danaine butterflies." *Trans. Ent. Soc. Lond.* p. 152.

(113) —— (1919 a). "Butterfly vision." *Trans. Ent. Soc. Lond.* p. 1.

(114) —— (1919 b). "On the histology of the scent-organs in the genus *Hydroptila* Dal." *Trans. Ent. Soc. Lond.* p. 420.

(115) —— (1923). "On the tympanic organ in *Chrysiridia ripheus* Dru." *Trans. Ent. Soc. Lond.* p. 443.

(116) —— (1925 a). "On the abdominal brushes in certain male noctuid moths." *Trans. Ent. Soc. Lond.* p. 1.

(117) ELTRINGHAM, H. (1925 *b*). "On the abdominal glands in *Heliconius* (Lepidoptera)." *Trans. Ent. Soc. Lond.* p. 269.

(118) —— (1925 *c*). "On the source of the sphragidal fluid in *Parnassius apollo* (Lep.)." *Trans. Ent. Soc. Lond.* p. 11.

(119) —— (1926 *a*). "On the abdominal glands in *Colaenis, Dione* and *Eueides*." *Trans. Ent. Soc. Lond.* p. 263.

(120) —— (1926 *b*). "On the structure of an organ in the hind-wing of *Myrmeleon nostras* Fourc." *Trans. Ent. Soc. Lond.* p. 267.

(121) EMERY, C. (1884). "Untersuchungen ueber *Luciola italica*." *Zeitschr. f. Wiss. Zool.* **40**, 338.

(122) ENGLEHARDT, V. von (1914). "Hancocksche Druese von *Oecanthus pellucens* Scop." *Zool. Anz.* **44**, 219.

(123–26) FABRE, J. H. *Souvenirs entomologiques*. Paris. 5me sér. 1897 (= 123). 6me sér. 1899 (= 124). 9me sér. 1907 (= 125). 10me sér. 1910 (= 126).

(127) FEUERBORN, H. J. (1922 *a*). "Das Hypopygium 'inversum' und 'circumversum' der Dipteren." *Zool. Anz.* **55**, 189.

(128) —— (1922 *b*). "Der sexuelle Reizapparat (Schmuck-, Duft-, und Beruehrungsorgane) der Psychodiden, etc." *Arch. f. Naturges.* **88**, Heft 4, 1.

(129) FOWLER, W. W. (1889–91). *The Coleoptera of the British Isles*. Vol. 2, 1888. Vol. 3. 1889. Vol. 4. 1890. Vol. 5, 1891.

(130) FRAUSTORFER, H. (1921). "Die Orthoptera der Schweiz." *Arch. f. Naturges.* **87**, Heft 5, 1.

(131) FREILING, H. (1909). "Duftorgane der weiblichen Schmetterlingen nebst Beitraegen zur Kenntnis der Sinnesorgane auf den Schmetterlingsfluegel und der Duftpinsel der Maennchen von *Danais* und *Euploea*." *Zeitschr. f. Wiss. Zool.* **92**, 210.

(132) FULTON, B. B. (1915). "The Tree-crickets of New York. Life-history and Bionomics." *Techn. Bull.* **42** *N.Y. Agric. Exper. Sta.* p. 1.

(133) GADEAU DE KERVILLE (1903). "L'accouplement des Forficulides." *Bull. Soc. Ent. France*, p. 85.

(134) —— (1906). "Sur l'accouplement et les œufs d'*Anisolabis mauretanica* H. Lucas." *Bull. Soc. Ent. France*, p. 202.

(135) GAHAN, C. J. (1889). "Note on the variation of the mandibles in the males and descriptions of the females of the Prionidous genera *Priotyrannus* and *Cacosceles*." *Ann. Mag. Nat. Hist.* 6 ser. **4**, 374.

(136) —— (1918). "The deathwatch, notes and observations." *Entomologist*, **51**, 121, 153.

(137) GARMAN, H. (1891). "On a singular gland possessed by the male *Hadenoecus subterraneus*." *Psyche*, **6**, 105.

(138) GATES, F. C. (1917). "Synchronism in the flashing of fireflies." *Science*, N.S. **46**, 314.

(139) GEIPEL, E. (1915). "Beitraege zur Anatomie der Leuchtorgane tropischer Kaefer." *Zeitschr. f. Wiss. Zool.* **112**, 239.

(140) GERHARDT, U. (1913). "Copulation und Spermatophoren von Grylliden und Locustodeen." Teil 1. *Zool. Jahrb. Syst.* **35**, 415.

(141) —— (1914). The same. Teil 2. *Zool. Jahrb. Syst.* **37**, 1.

(142) —— (1921). "Neue Studien ueber Copulation und Spermatophoren von Grylliden und Locustodeen." *Acta Zool.* **2**, 293.

(143) GERMER, F. (1912). "Untersuchungen ueber den Bau und die Lebensweise der Lymexeloniden, etc." *Zeitschr. f. Wiss. Zool.* **101**, 683.

(144) GILLMER, M. (1922). "Mitteilungen zur Entwicklungsgeschichte des *Biston hirtarius* Cl." *Int. Ent. Zeitschr.* **16**, 34, 40.

(145) GLASER, R. W. (1923). "The effect of food on longevity and reproduction in flies." *Journ. Exp. Zool.* **38**, 383.

(146) GOETGHEBUER, M. (1914). "Notes à propos de l'accouplement de *Johannseniella* Will. (Ceratopogon Mg.), *nitida* Mcq." *Ann. Soc. Ent. Belge*, **58**, 202.

(147) —— (1922). "Nouveaux matériaux pour l'étude de la faune des Chironomides de Belgique. (Diptera)." *Ann. Biol. Lacustre*, **11**, 38.

(148) GOLDSCHMIDT, R. (1923). *The mechanism and physiology of sex determination*. (Trans. W. J. Dakin.) London.

(149) GORDON, R. M. (1922). "Notes on the bionomics of *Stegomyia calopus* Meigen, in Brazil." *Ann. Trop. Med. and Parasitol.* **16**, 431.

(150) GORHAM, H. S. (1880). "On the structure of the Lampyridae, with reference to phosphorescence." *Trans. Ent. Soc. Lond.* p. 63.

(151) GREEN, E. E. (1899). "Notes on the assembling of males of certain moths in Ceylon." *Ent. Mo. Mag.* **35**, 258.

(152) —— (1912). "On some luminous Coleoptera from Ceylon." *Trans. Ent. Soc. Lond.* p. 717.

(153) —— (1921). "Notes on the habits of the bee, *Anthidium manicatum*." *Proc. Ent. Soc. Lond.* 5 Oct. p. lxxii.

(154) GROSVENOR, T. H. L. (1921). "Race crosses in *Zygaena*." *Proc. Ent. Soc. Lond.* 5 Oct. p. lxxiii.

(155) GRUHL, K. (1924). "Paarungs Gewohnheit der Dipteren." *Zeitschr. f. Wiss. Zool.* **122**, 205.

(156) GUDGER, E. W. (1919). "A historical note on the synchronous flashing of fireflies." *Science,* N.S. **50**, 188.

(157) HAASE, E. (1888). "Dufteinrichtungen indischer Schmetterlingen." *Zool. Anz.* **11**, 475.

(158) HAETTICH, E. (1907). "Ueber den Bau der rudimentaeren Mundwerkezeuge bei Sphingiden und Saturniden." *Zeitschr. f. Wiss. Insektenbiol.* **3**, 229, 261.

(159) HAMM, A. H. (1908). "Observations on *Empis livida* L." *Ent. Mo. Mag.* **44**, 181.

(160) —— (1909 a). "Observations on *Empis opaca* F." *Ent. Mo. Mag.* **45**, 132.

(161) —— (1909 b). "Further observations on the Empinae." *Ent. Mo. Mag.* **45**, 157.

(162) —— (1919). "A ribbon-making fly: the oviposition of *Ceratopogon nitidus* Macq." *Ent. Mo. Mag.* **55**, 66.

(163) HAMM, A. H. and RICHARDS, O. W. (1926). "The biology of the British Crabronidae." *Trans. Ent. Soc. Lond.* p. 297.

(164) HAMPSON, G. F. "Stridulation in certain Lepidoptera, and on the distortion of the hind-wings in the males of certain Ommatophorinae." *Proc. Zool. Soc. Lond.* p. 188.

(165) HANCOCK, J. L. (1905). "Habits of the striped meadow cricket (*Oecanthus fasciatus* Fitch)." *Amer. Nat.* **39**, 1.

(166) HANDSCHIN, E. (1926). *See* SCHULZE, P. (1926).

(167) HARNISCH, O. (1915). "Ueber den maennliche Begattungsapparat einiger Chrysomeliden." *Zeitschr. f. Wiss. Zool.* **114**, 1.

(168) HARRISON, J. W. H. (1913). "The hybrid Bistoninae." Oberthuer's *Étude de Lépidoptérologie comparée*, Fasc. VII, p. 343.

(169) —— (1918). "The pairing habits of certain bees." *Ent. Record*, **30**, 11.

(170) HASE, A. (1918). "Beobachtungen ueber den Kopulationsvorgang der Bettewanze (*Cimex lectularius* L.)." *Sitzungsber. Ges. Naturforsch. Freunde, Berlin*, p. 311.

(171) —— (1919). "Beitraege zur morphologischen und biologischen Kenntnis der Schlupfwespe *Lariophagus distinguendus* (Foerst.)." *Sitzungsber. Ges. Naturforsch. Freunde, Berlin*, p. 402.

(172) HAUSER, G. (1880). "Physiologischer und histologischer Untersuchungen ueber das Geruchs-organe der Insekten." *Zeitschr. f. Wiss. Zool.* **34**, 367.

(173) HEGNER, R. W. (1908). "Observations on the breeding habits of the Chrysomelid beetles, *Calligrapha bigsbyana*, *C. multipunctata*, and *C. lunata*." *Psyche*, **15**, 21.

(174) HEINER, H. (1914). "Zur Biologie und Anatomie von *Chloeon dipterum* L., *Baetis binoculatus* L., und *Habrophlebia fusca* Curt." *Jenaische Zeitschr. f. Naturwissenschaft*, **53** (2), 287.

(175) HENDEL, F. (1913). "Die Gattung *Platystoma* Meigen (Dipt.)." *Zool. Jahrb. Syst.* **35**, 55.

(176) HERING, M. (1926). *Biologie der Schmetterlinge*. Berlin.

(177) HESS, W. N. (1920). "Notes on the biology of some common Lampyridae." *Biol. Bull.* **38**, 39.

(178) HEWITT, C. G. (1906). "Some observations on the reproduction of the Hemiptera-Crypto-cerata." *Trans. Ent. Soc. Lond.* p. 86.

(179) HIRT, O. (1910). "Dufteinrichtungen der Neotropiden." *Zool. Jahrb. Anat.* **30**, 603.

(180) HOFFMANN, W. A. (1924). "The presence of an eversible gland in a midge." *Proc. Ent. Soc. Washington*, **26**, 144.

(181) HOLLAND, W. J. (1922). "*Calopteryx maculata*, Beauvois. An interesting photograph." *Proc. Ent. Soc. Washington*, **24**, 117.

(182) HOPKINS, A. D. (1894). "Sexual characters in the Scolytidae." *Canad. Ent.* **26**, 274.

(183) —— (1898). "On the life history and habits of the 'wood-engraver' ambrosia beetle—*Xyleborus xylographus* (Say.), *saxesini* (Ratz.)." *Canad. Ent.* **30**, 21.

(184) HOUGHTON, C. O. (1909). "Observations on the mating habits of *Oecanthus*." *Ent. News*, **20**, 274.

(185) HOWARD, L. O. (1886). "The excessive voracity of the female mantis." *Science*, **8**, 326.

(186) HOWARD, L. O., DYAR, H. G. and KNAB, F. (1912). "The mosquitoes of North and Central America and the West Indies." *Carnegie Institute, Washington*. 1. A general consideration of Mosquitoes, their habits, and relation to the human species.

(187) HOWLETT, M. (1907). "Note on the coupling of *Empis borealis*." *Ent. Mo. Mag.* **43**, 229.

(188) —— (1912). "The effect of oil of Citronella on two species of *Dacus*." *Trans. Ent. Soc. Lond.* p. 412.

(189) —— (1915). "Chemical reactions of fruitflies." *Bull. Ent. Research*, **6**, 297.

(190) HUBBARD, H. G. (1896). "Ambrosia beetles." *U S. Dept. Agric. Yearbook*, p. 421.

(191) —— (1897). "The ambrosia beetles of the United States." *U.S. Dept. Agric. Div. Ent. Bull.* N.S. **7**, 9.

(192) HUDSON, G. H. (1918). "Concerted flashing of fireflies." *Science*, N.S. **48**, 573.

(193) —— (1920). "On some examples of New Zealand insects illustrating the Darwinian principle of Sexual Selection." *Trans. N.Z. Inst.* **52**, 431.

(194) HUDSON, G. V. (1921). "Pairing of *Bombus terrestris* in New Zealand." *Ent. Mo. Mag.* **57**, 65.

(195) HUXLEY, J. S. (1923). "Courtship activities in the Redthroated Diver (*Colymbus stellatus* Pontopp.) together with a discussion of the evolution of courtship in birds." *Journ. Linn. Soc. Zool.* **35**, 253.

(196) —— (1924). "Constant differential growth-ratios and their significance." *Nature*, **114**, 895.

(197) ILLIG, K. (1902). "Duftorgane der maennlicher Schmetterlinge." *Zoologica*, **15**, Heft 38, 1.

(198) ILLINGWORTH, J. F. (1917). "Notes on the mating of cockroaches." *Proc. Hawai. Ent. Soc.* **3**, 374.

(199) JACOBI, A. (1907). "Ein Schrillapparat bei Singcikaden." *Zool. Anz.* **32**, 67.

(200) JENSEN, I. P. (1909). "Courting and mating of *Oecanthus fasciatus* Harris." *Canad. Ent.* **41**, 25.

(201) JOHANNSEN, F. (1921). "Insect life on the western arctic coast of America." *Rep. Canad. Arctic Expedition*, 1913–18, **3**, Part K, Ottawa.

(202) JORDAN, K. (1921). "Stridulating organs in *Holocera*, etc. (Saturniidae)." *Proc. Ent. Soc. Lond.* 2 Feb. p. ii.

(203) JOY, N. H. (1902). No title. (Fighting of males of *Apatura iris* L.) *Proc. Ent. Soc. Lond.* 19 Nov. p. xl.

(204) KELLOGG, V. (1894). "The taxonomic value of the scales in the Lepidoptera." *Kansas Univ. Quarterly*, **3**, 45.

(205) —— (1906). "A note on assortative mating." *Science*, N.S. **24**, 665.

(206) KENNEDY, A. (1838). "Observations upon the economy of several species of Hymenoptera found in a London garden." *Philosoph. Mag.* **12**, 14.

(207) KIEFFER, J. J. (1925). *Faune de France, Chironomidae Ceratopogoninae.* Paris.

(208) KIRK, H. B. (1923). "Notes on the mating habits and early life-history of the Culicid, *Opifex fuscus* Hutton." *Trans. New Zealand Inst.* **54**, 400.

(209) KLATT, B. (1913). "Experimentelle Untersuchungen ueber die Beziehungen zwischen Copulation und Eiablage beim Schwammspinner." *Biol. Centralbl.* **33**, 620, 629.

(210) —— (1920). "Beitraege zur Sexualphysiologie des Schwammspinners." *Biol. Zentrbl.* **40**, 539.

(211) KNAB, F. (1905). "Observations on Lampyridae." *Canad. Ent.* **37**, 238.

(212) —— (1909). "Nuptial colours of the Chrysomelidae." *Proc. Ent. Soc. Washington*, **11**, 151.

(213) KOEHLER, F. (1900). "Die Duftschuppen der Gattung *Lycaena*." *Zool. Jahrb. Syst.* **13**, 105.

(214) KONCEK, S. K. (1924). "Zur Histologie der Rueckendruesen unserer einheimischen Blattiden." *Zeitschr. f. Wiss. Zool.* **122**, 311.

(215) KRAUSS, H. (1890). "Die Duftdruese der *Aphlebia bivittata* Brullé (Blattidae) von Teneriffa." *Zool. Anz.* **13**, 584.

(216) —— (1891). "Beitrag zur Kenntis Westafrikanischer Orthopteren. I." *Zool. Jahrb. Syst.* **5**, 344.

(217) KRUEGER, E. (1921). "Das Geraeusch der *Ageronia*-Arten." *Ent. Rundsch.* **38**. 35.

(218) LABAUME, W. (1909). "Ueber die Metamorphose der Ephemeriden." *Sitzungsber. Ges. Naturforsch Freunde, Berlin*, p. 137.

(219) LABITTE, A. (1919). "Observations sur *Rhodocera rhamni*." *Bull. Mus. Hist. Nat. Paris*, **25**, 624.

(220) LAMB, C. G. (1922). "The geometry of insect pairing." *Proc. Roy. Soc.* B, **94**, 1.

LAMBORN, W. A. (All dates). *See* POULTON, E. B.

(221) LECERF, F. (1920). "Probable heteromorphism of secondary sexual characters in *Triglochana*." *Proc. Ent. Soc. Lond.* 17 Nov. p. lxxxiv.

(222) LENGERKEN, H. von (1924). *See* SCHULZE, P. (1924).

(223) LESNE, P. (1901). "La variation sexuelle chez les mâles de certains Coléoptères appartenant à la famille des Bostrychides etc." *Compt. Rend. Acad. Sci. Paris*, **132**, 847. (Errata, p. 896.)

(224) —— (1921). "Observations sur deux espèces de Tipulides." *Bull. Mus. Hist. Nat. Paris*, **27**, 302.

(225) LIANG, SHUWEN (1925). "Morphologie des Hypopygium, der maennliche Genitaldruesen und des Verdauungssystems von *Thaumastoptera calceata* Mik. (Tipulidae, Diptera)." *Arch. f. Naturges.* **91**, Heft 1, 1.

(226) LINDNER, E. (1923). *See* SCHULZE, P. (1923).

(227) LONGSTAFF, G. B. (1904). No title. (The scents of Indian Pierines.) *Proc. Ent. Soc. Lond.* 7 Dec. p. lxxxviii.

(228) —— (1905 *a*). No title. (Scent of *Gonepteryx*.) *Proc. Ent. Soc. Lond.* 7 June, p. xxxv.

(229) —— (1905 *b*). "On the scents of some common English butterflies." *Ent. Mo. Mag.* **41**, 112.

(230) —— (1912). *Butterfly Hunting in Many Lands.* London.

(231) LOWNE, B. T. (1871). "Observations on immature sexuality and alternate generations in insects." *Trans. Ent. Soc. Lond.* p. 193.

(232) LUBBOCK, J. (1871). "A monograph of the Collembola and Thysanura." *Ray Soc. London*, p. 109.

(233) LUNDBECK, W. (1910). *Diptera Danica. 3. Empididae.* Copenhagen.

(234) —— (1912). *Diptera Danica. 4. Dolichopodidae.* Copenhagen.

(235) MACATEE, W. L. (1909). "Some habits of the Empididae." *Ent. News*, **20**, 359.

(236) MacDERMOTT, F. A. (1910). "A note on the light emission of American Lampyridae." *Canad. Ent.* **42**, 357.

(237) —— (1911). "Some further observations on the light emission of American Lampyridae." *Canad. Ent.* **43**, 399.

(238) —— (1912 a). "Observations on the light emission of American Lampyridae." *Canad. Ent.* **44**, 309.

(239) —— (1912 b). "Observations on the light emission of American Lampyridae; notes and corrections." *Canad. Ent.* **44**, 73.

(240) —— (1916). "Flashing of fireflies." *Science*, N.S. **44**, 610.

(241) MACGILL, E. I. (1922). "The life-history of *Aphidius avenae* (Hal.), a Braconid parasitic on the nettle aphis (*Macrosiphum urticae*)." *Proc. Roy. Phys. Soc. Edinburgh*, **43**, 51.

(242) MACNAMARA, C. (1926). "The 'drumming' of stoneflies (Plecoptera)." *Canad. Ent.* **58**, 53.

(243) MANDERS, N. (1908). "The courtship of *Hepialus humuli*." *Ent. Record*, **20**, 202.

(244) MARSHALL, G. A. K. (1902). "Five years' observations on the bionomics of South African insects, etc." *Trans. Ent. Soc. Lond.* p. 287. (p. 538 Courtship of *Limnas chrysippus*.)

(245) MAYER, A. G. (1900). "The mating instincts of moths." *Ann. Mag. Nat. Hist.* **5**, 7th ser. p. 183.

(246) MAYER, A. G. and SOULE, C. G. (1906). "Reactions of caterpillars and moths." *Journ. Exp. Zool.* **3**, 415.

(247) MAYER, A. M. (1874). "Experiments on the supposed auditory apparatus of the mosquito." *Amer. Nat.* **8**, 577.

(248) MEISENHEIMER, J. (1921). *Geschlecht und Geschlechter*. Band **1**. Jena.

(249) MELANDER, A. L. (1902). "Monograph of the North American Empididae." *Trans. Amer. Ent. Soc.* **28**, 195.

(250) MELIN, D. (1923). "Contributions to the knowledge of the biology, metamorphosis and distribution of the Swedish Asilids." *Zoologiska Bidrag från Uppsala*.

(251) MERCIER, L. (1914). "Caractère sexuel secondaire chez les Panorpes. Le rôle des glandes salivaires des mâles." *Arch. Zool. Exp. et Gén.* **55**, 1.

(252) MERRITT-HAWKES, O. A. (1920). "Observations on the life-history, biology and Genetics of the Ladybird beetle, *Adalia bipunctata* Muls." *Proc. Zool. Soc. Lond.* **2**, 475.

(253) —— (1923). "The hibernation of Coccinellids on mountains." *Ent. Mo. Mag.* **59**, 53.

(254) MERTENS, H. (1923). "Biologische und morphologische Untersuchungen an Plekopteren." *Arch. f. Naturges* **89**, Heft 2, 1.

(255) METCALF, C. L. (1921). "The genitalia of male Syrphidae, etc." *Ann. Ent. Soc. Amer.* **14**, 169.

(256) MIALL, L. C. and DENNY, A. (1886). *The Structure and Life-history of the Cockroach*. London.

(257) MICHAEL, H. (1923). "Ueber den Bau der Geschlechtsapparat und die Kopulation von *Bombyx mori*." *Arch. f. Naturges*. **89**, Heft 12, 25.

(258) MILLER, D. (1923). "Material for a monograph on the Diptera fauna of New Zealand. Part 6. Empididae." *Trans. New Zealand Inst.* **54**, 440.

(259) MINCHIN, E. A. (1890). "Further observations on the dorsal gland in the abdomen of *Periplaneta* and its allies." *Zool. Anz.* **13**, 41.

(260) —— (1905). "Report on the anatomy of the Tsetse fly (*Glossina palpalis*)." *Proc. Roy. Soc.* B, **76**, 531.

(261) MORGAN, T. H. (1919). "The genetic and operative evidence relating to secondary sexual characters." *Carnegie Inst. Washington*.

(262) MORICE, F. D. (1912). "The secondary sexual characters of Aculeate Hymenoptera. Presidential address." *Proc. Ent. Soc. Lond.* 15 Jan. (1913) p. clxix.

(263) —— (1919). "Androconia in a bee." *Proc. Ent. Soc. Lond.* 4 June, p. xlii.

(264) —— (1921). "British Hymenoptera." *Proc. Ent. Soc. Lond.* 1 June, p. lxix.

(265) MORSE, E. S. (1916 a). "Fireflies flashing in unison." *Science*, N.S. **43**, 169.

(266) —— (1916 b). "Fireflies flashing in unison." *Science*, N.S. **44**, 387.

(267) —— (1924). "The synchronous flashing of fireflies." *Science*, N.S. **59**, 163.

(268) MORSE, F. (1918). "Fireflies flashing in unison." *Science*, N.S. **48**, 418.

(269) MOSELY, H. N. (1899). *Notes by a Naturalist on the "Challenger."* London. (p. 373.)

(270) MOSELY, M. E. (1919). "Scent organs in the genus *Hydroptila* (Trichoptera)." *Trans. Ent. Soc. Lond.* p. 393.

(271) —— (1922). "Scent organs in New Zealand Trichoptera." *Proc. Ent. Soc. Lond.* 15 Nov. p. civ.

(272) —— (1923). "Scent organs on the genus *Hydroptila* (Trichoptera)." *Trans. Ent. Soc. Lond.* p. 291.

(273) MUELLER, F. *See* LONGSTAFF, G. B. (1912). (Collected papers on the scent-organs of Lepidoptera in the appendix.)

(274) MUELLER, W. (1887). "Duftorgane bei Phryganiden." *Arch. f. Naturges.* **53**, 95.

(275) MUIR, F. (1912). *See* SHARP, D. (1912).

(276) —— (1919). "On the mechanism of the male genital tube in Coleoptera." *Trans. Ent. Soc. Lond.* p. 404.

(277) NEWPORT, G. (1851). "On the anatomy and affinities of *Pteronarcys regalis* Newm. etc." *Trans. Linn. Soc. Zool.* **20**, 425.

(278) NICHOLSON, G. (1926). "*Gastrophilus equi* and other insects on mountain tops." *Ent. Mo. Mag.* **62**, 99.

(279) NIEDEN, F. (1907). "Der sexuelle Dimorphismus der Antennen bei den Lepidopteren." *Zeitschr. f. wiss. Insektenbiologie*, **3**, 137, 197, 272, 293, 325.

(280) NONIDEZ, J. (1920). "The internal phenomena of reproduction in *Drosophila*." *Biol. Bull.* **39**, 207.

(281) OSTEN-SACKEN, C. R. (1895). "Contributions to the study of the Liponeuridae." *Berlin. Ent. Zeitschr.* **40**, 148.

(282) PACKARD, A. S. (1898). *A Textbook of Entomology.* London.

(283) PAIVA, C. A. (1919). "Notes on the Indian glow-worm *Lamprophorus tenebrosus* (Walk.)." *Records of the Indian Mus.* **16**, 19.

(284) PECKHAM, E. G. and G. W. (1905). *Wasps, Social and Solitary.* London.

(285) PÉREZ, J. (1911). "Sur quelques particularités curieuses du rapprochement des sexes chez certaines Diptères." *Bull. Sci. France et Belg.* **45**, 1.

(286) PERINGUEY, L. (1884). "First contribution to the South African Coleopterous fauna." *Trans. S. Afric. Philos. Soc.* **3**, 174.

(287) PERKINS, R. C. L. (1918). *See* POULTON, E. B. (1918 c).

(288) PETERSEN, W. (1892). "Ungleichzeit in der Erscheinung der Geschlechter bei Schmetterlingen." *Zool. Jahrb. Syst.* **6**, 71.

(289) —— (1907 a). "Ueber die Spermatophoren der Schmetterlinge." *Zeitschr. f. Wiss. Zool.* **88**, 117.

(290) —— (1907 b). "Ein Beitrag zur Frage der geschlechtlichen Zuchtwahl (Lepidopteren)." *Biol. Centralbl.* **27**, 427.

(291) PETRUNKEWITSCH, A. und GUAITA, G. von (1901). "Ueber den geschlechtlichen Dimorphismus bei Tonapparat der Orthopteren." *Zool. Jahrb. Syst.* **14**, 291.

(292) PIERCE, F. N. (1909). *The Genitalia of the Group Noctuidae of the Lepidoptera of the British Islands.* Liverpool.

(293) PIERSOL, W. H. (1907). "The curious mating habits of the fly *Rivellia boscii*." *Amer. Nat.* **41**, 465.

(294) PLANTA, A. von (1888). "Ueber der Futtersaft der Bienen." *Zeitschr. f. Physiol. Chemie.* **12**, 327, 555.

(295) POISSON, R. (1924). "Contribution à l'étude des Hémiptères Aquatiques." *Bull. Sci. France Belg.* **58**, 49.

(296) POLJANEC, L. (1902). "Morphologie der aeusseren Geschlechtsorgane bei den maennlichen Lepidopteren." *Arb. Zool. Inst. Wien*, **13**, 155.

(297) POULTON, E. B. (1890). "The external morphology of the Lepidopterous pupa etc." *Trans. Linn. Soc. Zool.* 2nd ser. **5**, 187.

(298) —— (1896). "On the courtship of certain European Acridiidae." *Trans. Ent. Soc. Lond.* p. 233.

(299) —— (1904 a). No title. (Egg-laying and courtship of *Vanessa urticae*.) *Proc. Ent. Soc. Lond.* 1 June, p. xli.

(300) —— (1904 b). No title. (Insects on mountain tops.) *Proc. Ent. Soc. Lond.* 16 Mar. p. xxii.

(301) —— (1906). "Predaceous insects and their prey." *Trans. Ent. Soc. Lond.* p. 323.

(302) —— (1911). "Observations on the courtship of *Planema alcinoe* Feld." (Lamborn's observations.) *Proc. Ent. Soc. Lond.* 6 Dec. p. xcv.

(303) —— (1912). "The anal tufts of *Glutaphrissa saba* extruded in courtship." *Proc. Ent. Soc. Lond.* 7 Feb. p. v.

(304) —— (1913 a). "Mr W. A. Lamborn's observations on the courtship of a Lycid beetle." *Proc. Ent. Soc. Lond.* 15 Oct. p. lxxiv and 5 Nov. p. lxxxviii.

(305) —— (1913 b). "Mr W. A. Lamborn's observations on marriage by capture in a W. African wasp." *Rep. Brit. Assoc. Adv. Sci.* 1913, p. 511.

(306) —— (1913 c). "Empidae and their prey in relation to courtship." *Ent. Mo. Mag.* **49**, 177.

(307) —— (1914). "Observation of the epigamic use of the anal brushes of the male *Amauris psyttalea* Ploetz." (Carpenter's observations.) *Proc. Ent. Soc. Lond.* 2 Dec. p. cxi.

(308) —— (1918 a). "The relation of the anal tufts to the brands in the hind-wings observed and the scent perceived in a male Danaine butterfly." (Lamborn's observations.) *Proc. Ent. Soc. Lond.* 4 Dec. p. clxxii.

(309) POULTON, E . B. (1918 *b*). "Capt. G. D. H. Carpenter's further notes on ex-German S.E. Africa, almost exclusively east of Lake Tanganyika." *Proc. Ent. Soc. Lond.* 5 June, p. lxxxviii (see especially p. cxiii).

(310) —— (1918 *c*). "The pairing of *Stylops* and the assembling of the males observed by Dr R. C. L. Perkins." *Proc. Ent. Soc. Lond.* 1 May, p. lxxi.

(311) —— (1921 *a*). "An oriental Danaine butterfly brushing the brands on its hind wings." (Lamborn's observations.) *Proc. Ent. Soc. Lond.* 2 Nov., p. xcv.

(312) —— (1921 *b*). "The courtship of the Cicada *Monometopa insignis* Dist. (*Tibicinae*), observed in Tanganyika Territory." *Proc. Ent. Soc. Lond.* 1 June, p. lxiii.

(313) PRZIBRAM, H. (1907). "Lebensgeschichte der Gottesanbeterinnen." *Zeitschr. f. wiss. Insektenbiol.* **3**, 117, 146.

(314) QUAIL, A. (1903). "On the antennae of the Hepialidae (Lepidoptera, Jugatae)." *Trans. Ent. Soc. Lond.* p. 499.

(315) RABAUD, E. (1916). "Accouplement d'un mâle décapité de *Mantis religiosa.*" *Bull. Soc. Ent. France*, p. 57.

(316) RAMME, W. (1923). "Vorarbeiten zu einer Monographie des Blattidengenus *Ectobius* Steph." *Arch. f. Naturges.* **89**, Heft 7, 97.

(317) RAU, P. and N. (1912). "Longevity in Saturniid moths." *Journ. Exper. Zool.* **12**, 179.

(318) —— (1913). "The biology of *Stagmomantis carolina.*" *Trans. Acad. Sci. St Louis*, **22**, 58.

(319) —— (1918). *Wasp Studies Afield.* Princeton.

(320) RAU, P. (1924). "A note on the courtship of *Telea polyphemus.*" *Canad. Ent.* **56**, 271.

(321) REINKING, O. A. (1921). "The synchronal flashing of fireflies." *Science*, N.S. **53**, 485.

(322) RICHARDS, O. W. (1924). "The mating habits of certain species of *Micropteryx.*" *Ent. Mo. Mag.* **60**, 31.

(323) RIESEN, H. (1909). No title. (Die Begattung von *Perla marginata* Panz.) *Berlin. Ent. Zeitschr.* **54**, 37.

(324) RILEY, C. V. and HOWARD, H. O. (1892–3). "The female rearhorse versus the male." *Insect Life*, **5**, 145.

(325) ROBSON, J. E. (1887 *a*). "On the flight and pairing of *Hepialus hectus* and *humuli.*" *Ent. Mo. Mag.* **23**, 186.

(326) —— (1887 *b*). "On the flight and pairing of *Hepialus sylvinus* and *lupulinus.*" *Ent. Mo. Mag.* **23**, 214.

(327) —— (1891). "The flight and pairing of the genus *Hepialus.*" *Ent. Mo. Mag.* **27**, 197.

(328) SAMAL, J. (1923). "Étude morphologique et biologique de *Perla abdominalis* Burm. (Plécoptère)." *Ann. Biol. Lacustre*, **12**, 229.

(329) SAUNDERS, E. (1909). "*Bombi* and other Aculeates collected in 1908 in the Berner Oberland by the Rev. A. E. Eaton, M.A." *Ent. Mo. Mag.* **45**, 83.

(330) SAUNDERS, L. G. (1924). "On the life-history and the anatomy of the early stages of *Forcipomyia* (Diptera, Nematocera, Ceratopogoninae)." *Parasitology*, **16**, 164.

(331) SAUNDERS, S. S. (1880). No title. (Synchronous flashing of *Luciola italica.*) *Proc. Ent. Soc. Lond.* 4 Feb. p. ii.

(332) SCHOENEMUND, E. (1924). *See* SCHULZE, P. (1924).

(333) SCHULZE, P. (1923–6). (Unfinished.) *Biologie der Tiere Deutschlands.* Herausgegeben von Dr P. Schulze. Berlin.

(334) Lief. 5, Teil. 38. Diptera. Lindner, E. 1923.

(335) Lief. 9, Teil. 34. Ephemeroptera. Ulmer, G. 1924.

(336) Lief. 10, Teil. 32. Plecoptera. Schoenemund, E. 1924.

(337) Lief. 12, Teil. 40. Coleoptera. Lengerken, H. von. 1924.

(338) Lief. 13, Teil. 36. Trichoptera. Ulmer, G. 1925.

(339) Lief. 20, Teil. 25. Collembola. Handschin, E. 1926.

(340) Lief. 21, Teil. 35. Mecaptera. Stitz, H. 1926.

(341) SCOTT, HUGH (1922). "*Sirex gigas*; early appearance and other habits." *Ent. Mo. Mag.* **59**, 113.

(342) —— (1926 *a*). "Note on the swarming of gnats and midges round lofty towers." *Ent. Mo. Mag.* **62**, 18.

(343) —— (1926 *b*). "*Sirex gigas, Gastrophilus equi*, and other insects on bare mountain tops." *Ent. Mo. Mag.* **62**, 19.

(344) SÉGUY, E. (1923). *Faune de France, Diptères Anthomyides.* Paris.

(345) SEITZ, A. (1894). "Allgemeine Biologie der Schmetterlinge. 3. Fortpflanzung." *Zool. Jahrb. Syst.* **7**, 823.

(346) —— (1913). "On the sense of vision in insects." *Rep.* 2, *Int. Ent. Congress, Oxford*, p. 198.

(347) SHARP, D. (1880–2). "On aquatic carnivorous Coleoptera or Dytiscidae." *Sci. Trans. Roy. Dublin Soc.* 2 ser. **2**, 1.

(348) SHARP, D. and MUIR, F. (1912). "The comparative anatomy of the male genital tube in Coleoptera." *Trans. Ent. Soc. Lond.* p. 477.

(349) SHULL, A. F. (1907). "The stridulation of the Snowy Tree-cricket (*Oecanthus niveus*)." *Canad. Ent.* **39**, 213.

(350) SIMMERMANN, G. (1884). "Haftapparat an Tarsalgliedern von Insekten." *Zeitschr. f. Wiss. Zool.* **40**, 481.

(351) SINGH PRUTHI, H. (1925). "The morphology of the male genitalia in Rhynchota." *Trans. Ent. Soc. Lond.* p. 127.

(352) SKERTCHLY, S. B. J. (1889). "On the habits of certain Bornean butterflies." *Ann. Mag. Nat. Hist.* (6), **4**, 209.

(353) SKINNER, H. and WILLIAMS, R. C. (1922). "On the male genitalia of the larger Hesperidae of North America." *Ann. Ent. Soc. Amer.* **48**, 109, 283.

(354) SLADEN, F. W. L. (1912). *The Humblebee and how to domesticate it.* London.

(355) SMITH, K. M. (1919). "A comparative study of certain sense-organs in the antennae and palpi of Diptera." *Proc. Zool. Soc. Lond.* p. 31.

(356) SMITH, L. W. (1918). "Studies of N. American Plecoptera (Pteronarcinae and Perlodinae)." *Trans. Amer. Ent. Soc.* **43**, 433.

(357) SMITH, R. C. (1922). "The biology of the Chrysopidae." *Cornell Univ. Agric. Exp. Sta. Mem.* **58**, 1291.

(358) SNODGRASS, R. E. (1902). "The inverted hypopygium of *Dasyllis* and *Laphria*." *Psyche*, **9**, 399.

(359) SNYDER, C. D. and A. v. 't. H. (1920). "The flashing interval of fireflies. Its temperature coefficient; an explanation of synchronous flashing." *Amer. Journ. Physiol.* **51**, 536.

(360) SOLOWIOW, P. (1924). "Biologische Beobachtungen ueber Holzlaeuse (*Atropos pulsatoria* L.)." *Zool. Anz.* **59**, 238.

(361) SOULE, C. G. (1902). "Notes on the hybrids of *Samia cynthia* and *Attacus promethea*." *Psyche*, **9**, 411.

(362) STEIN, P. (1920). "Zur Biologie *Ctenophora atrata* L." *Zool. Jahrb. Syst.* **43**, 33.

(363) STITZ, H. (1926). *See* SCHULZE, P. (1926).

(364) STOBBE, R. (1912). "Die abdominalen Duftorgane der maennlichen Sphingiden und Noctuiden." *Zool. Jahrb. Anat.* **32**, 493.

(365) STROHMEYER, H. (1911). "Die biologische Bedeutung sekundaerer Geschlechtscharakter am Kopfe weiblicher Platypodiden." *Ent. Blaetter.* **7**, 103.

(366) STURTEVANT, A. H. (1915). "Experiments on sex recognition and the problem of sexual selection in *Drosophila*." *Journ. Anim. Behav.* **5**, 351.

(367) —— (1921). "The N. American species of *Drosophila*." *Carnegie Inst. Washington.*

(368) TILLYARD, R. J. (1912). "On some new and rare Australian Agrionidae (Odonata)." *Proc. Linn. Soc. New S. Wales*, **37**, 404.

(369) —— (1917). *The Biology of Dragonflies.* Cambridge.

(370) —— (1918). "Studies in Australian Neuroptera, No. 7. The life-history of *Psychopsis elegans*." *Proc. Linn. Soc. New S. Wales*, **43**, 814.

(371) TODD, J. E. (1885). "The flight of Robberflies in connection." *Amer. Nat.* **19**, 305.

(372) TONNOIR, A. (1919). "Notes sur les *Ptychopteridae* (Dipt.)—Description d'espèces nouvelles et d'un organe sexuel auxiliaire chez certaines mâles." *Ann. Soc. Ent. Belge*, **59**, 115.

(373) TULLOCH, F. (1906). "The internal anatomy of *Stomoxys*." *Proc. Roy. Soc.* B, **77**, 523.

(374) TURNER, C. L. (1916). "The breeding habits of Orthoptera." *Ann. Ent. Soc. Amer.* **9**, 117.

(375) —— (1923). "The Psychodidae as subjects for studies in breeding." *Amer. Nat.* **57**, 545.

(376) TURNER, H. J. (1916). "A note on the circumstances of pairing in some diurnal Lepidoptera." *Ent. Record*, **28**, 88.

(377) ULMER, G. (1924). *See* SCHULZE, P. (1924).

(378) —— (1925). *See* SCHULZE, P. (1925).

(379) URBAHN, E. (1913). "Abdominale Duftorgane bei weiblichen Schmetterlingen." *Jenaische Zeitschr. f. Naturw.* **50**, 277.

(380) VECK, H. L. van (1924). "The flashing of fireflies." *Science*, N.S. **59**, 379.

(381) VENOUR, S. (1906). "*Ephemera* (*danica* Muell.) male imago coupling with female subimago." *Ent. Mo. Mag.* **46**, 258.

(382) VERHOEFF, C. (1892). "Neue und wenig bekannte Gesetze aus der Hymenopterenbiologie." *Zool. Anz.* **15**, 362.

(383) VERRALL, G. H. (1909). *British Diptera, Stratiomyidae, etc.* London.

(384) VOGEL, R. (1921). "Zur Kenntnis der Geruchsorgane der Wespen und Bienen." *Zool. Anz.* **53**, 20.

(385) WAGNER, W. (1909). "Anlockung der Schlupfwespen-Maennchen durch Weibchen, die noch im Cocon sassen." *Zeitschr. f. wiss. Insektenbiol.* **5**, 245.

(386) WALKER, E. M. (1912). "The North American Dragonflies of the genus *Aeshna*." *Univ. Toronto Studies Biol. Ser.* No. 11, p. 1.

(387) —— (1915). "Notes on *Staurophlebia reticulata* Burm." *Canad. Ent.* **47**, 387.

(388) WALKER, F. W. (1919). "Synchronous movements in *Vanessa urticae* larvae, with notes on the attraction of certain male Lepidoptera by the females of their own species." *Psyche*, **26**, 13.

(389) WALKER, J. J. (1882). "Entomological collecting on a voyage in the Pacific." *Ent. Mo. Mag.* **19**, 22.

(390) WALSH, G. B. (1924). "*Sirex gigas* in elevated treeless places." *Ent. Mo. Mag.* **60**, 212.

(391) WARREN, B. C. S. (1920). "Some records of, and observations on, the flying-habit of butterflies when paired." *Ent. Record*, **32**, 218.

(392) WEBSTER, F. M. (1898). "Notes and observations on several species of Diptera." *Canad. Ent.* **30**, 18.

(393) WESENBURG-LUND, C. (1913). "Fortpflanzungsverhaeltnisse: Paarung und Eiablage der Suesswasserinsekten." *Fortschr. f. Naturwiss. Forsch.* **8**, 161.

(394) WHEELER, G. (1918). "Paired Lepidoptera in flight." *Ent. Record*, **30**, 152.

(395) WHEELER, W. M. (1889). "On two new species of Cecidomyid flies producing galls on *Antennaria plantaginifolia*." *Proc. Wis. N. H. S.* April, p. 209.

(396) WHEELER, W. M. and CHAPMAN, J. W. (1922). "The mating of *Diacamma*." *Psyche*, **29**, 203.

(397) WHEELER, W. M. (1924 a). *Social Life Amongst the Insects*. (p. 294, habits of *Scleroderma macrogaster* Ashm.) London.

(398) —— (1924 b). "The courtship of the Calobatas." *Journ. Heredity*, **15**, 485.

(399) WILLIAMS, C. B. (1922). "Co-ordinated rhythm in insects, with a record of sound production in an Aphid." *Entomologist*, **55**, 173.

(400) WILLIAMS, C. E. (1904). "Notes on the life-history of *Gongylus gongyloides* etc." *Trans. Ent. Soc. Lond.* p. 125.

(401) WILLIAMSON, E. B. (1905). "The Dragonflies (Odonata) of Burma and Lower Siam. 1. Subfamily Calopteryginae." *Proc. U.S. Nat. Mus.* **28**, 165.

(402) WITHYCOMBE, C. L. (1922 a) "Notes on the biology of some British Neuroptera (Planipennia)." *Trans. Ent. Soc. Lond.* p. 501.

(403) —— (1922 b). "On the life-history of *Boreus hiemalis* L." *Trans. Ent. Soc. Lond.* p. 312.

(404) —— (1926). "Additional remarks upon *Boreus hiemalis* L." *Ent. Mo. Mag.* **62**, 81.

(405) YERBURY, J. W. (1908). "A romantic tragedy in low life." *Ent. Mo. Mag.* **44**, 236.

(406) YOUNG, C. J. (1922). "Notes on the bionomics of *Stegomyia calopus* Meigen, in Brazil." *Ann. Trop. Med. and Parasitol.* **16**, 406.

20. INDEX.

To names of authors and of families, genera and species of insects mentioned in the text.

LES FORMES MINÉRALOGIQUES DU CALCAIRE CHEZ LES ÊTRES VIVANTS, ET LE PROBLÈME DE LEUR DÉTERMINISME

Par MARCEL PRENANT.

(Reçu le 14 juin, 1927.)

1. LES FORMES MINÉRALOGIQUES DU CALCAIRE.

Le carbonate de calcium, chacun le sait, se présente, dans la nature ou le laboratoire, sous deux formes minéralogiques principales, la calcite et l'aragonite. On sait moins que, outre ces deux formes, il en a été décrit un grand nombre d'autres. Ce sont[1]:

1° la calcite, ou $\beta - CaCO^3$;

2° la forme désignée par le symbole $\alpha - CaCO^3$;

3° l'aragonite, ou $\lambda - CaCO^3$;

4° la forme appelée $\mu - CaCO^3$ par Johnston, Merwin et Williamson;

5° la vatérite;

6° la ctypéite;

7° la conchite;

8° la lublinite;

9° le carbonate de calcium amorphe.

Valables ou non, ces formes, à l'exception peut-être de la dernière, selon Copisarow, correspondent au calcaire anhydre seul. Il faut donc leur ajouter les trois hydrates connus, qui ont respectivement pour formules

$$CaCO^3, 3H^2O; CaCO^3, 5H^2O; CaCO^3, 6H^2O.[2]$$
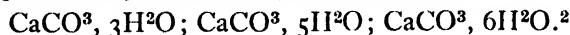
De ces douze formes, un certain nombre peuvent se présenter chez les êtres vivants; nous verrons plus loin que chez un animal donné, en un point donné, la forme minéralogique du calcaire qui se produit est toujours la même. Il existe donc un problème du déterminisme de la cristallisation du calcaire chez les êtres vivants.

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[1] Je ne fais qu'énumérer des formes très peu connues et douteuses, auxquelles on a donné les noms de pélagosite, thinolithe, pseudogaylussite, et jarrowite. Je renvoie, à leur sujet, à l'article de Leitmeier dans le traité de Doelter.

[2] Un dihydrate a été signalé par Kossmann dans la nature, mais il est peu connu.

Le but de cette revue est à la fois de poser les éléments de ce problème et d'indiquer la voie dans laquelle, selon moi, on doit chercher sa solution.

Nous n'aurons, heureusement, pas à considérer les douze formes énumérées. Certaines d'entre elles ont une autonomie plus ou moins douteuse, et méritent d'être rayées de la liste.

Il en est ainsi pour la conchite, que Kelly a décrite dans certaines coquilles de Mollusques, puis chez d'autres animaux, et même dans la nature minérale. Au dire de cet auteur, elle avait la plupart des caractères de l'aragonite, mais était optiquement uniaxe comme la calcite. Il est certain, depuis les critiques de Brauns et de Vater, que Kelly avait fait erreur dans ses observations optiques; Lacroix a reconnu la nature biaxe de "conchites" certaines, et tout le monde s'accorde à considérer la conchite comme de l'aragonite, ou tout au plus de l'aragonite poreuse. J'interpréterai toujours en ce sens les indications de Kelly que j'utiliserai.

La ctypéite, décrite par Lacroix, se présente en sphérolithes qui, selon Johnston, Merwin et Williamson, sont constitués par la forme μ. Meigen, et Linck, l'ont, d'autre part, identifiée à la vatérite. Avant la description de Lacroix, Sorby avait considéré ces mêmes sphérolithes comme formés d'aragonite, et c'est encore l'opinion, postérieure, de Vater. Lacroix, cependant, maintient que la ctypéite est une forme minéralogique distincte. Pour la lublinite, elle n'est, selon Quercigh, pas autre chose que de la calcite finement aciculaire; et Rinne est du même avis, après étude aux rayons X. Formes autonomes ou non, ces minéraux ne se rencontrent pas chez les êtres vivants, et il n'en sera plus question dans ce qui suit.

Pour certains auteurs, comme Johnston, Merwin et Williamson, et aussi Eitel, la vatérite et le calcaire amorphe sont, eux aussi, des calcites anormales, la première étant une calcite sphérolithique poreuse, et le second une calcite à grains très fins. Cette opinion est loin d'être généralement admise. Malgré les arguments très sérieux que l'on peut faire valoir pour identifier calcite et vatérite (il semble, entre autres, que les sphérolithes de vatérite forment, par leurs divers caractères physiques, une série continue dont les derniers termes ressemblent beaucoup aux sphérolithes de calcite), il apparaît comme probable, à l'heure actuelle, que la vatérite est bien une forme autonome: von Ohlshausen, et aussi Rinne, en effet, lui ont trouvé, aux rayons X, un spectre différent de celui de la calcite, et le dernier auteur qui s'est occupé de la vatérite, Heide, se prononce nettement pour son indépendance par rapport à la calcite, et affirme de plus que c'est bien une forme anhydre.

Nous n'avons pas d'arguments aussi sûrs en ce qui concerne le calcaire amorphe[1], mais l'opinion générale est en faveur de sa réelle isotropie, qu'on le regarde avec Copisarow comme un carbonate de calcium pentahydraté, ou avec les autres auteurs comme un carbonate anhydre. Il semble d'ailleurs que l'autonomie de la vatérite entraîne nécessairement celle du carbonate amorphe, puisque celui-ci est instable par rapport à la vatérite, elle-même instable par rapport à la calcite. En parlant, dans ce qui suit, de vatérite et de calcaire amorphe, je ne prétends cependant pas

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[1] On ne peut l'obtenir sec sans artifice, à cause de son instabilité. Bütschli le précipite en milieu fortement albumineux, et coagule aussitôt l'albumine à l'alcool, puis dessèche. Neuberg et Rewald font la précipitation en solution méthylique, où le calcaire amorphe est plus stable.

trancher la question, et ma principale raison d'agir ainsi est que, chez les animaux, calcaire amorphe et vatérite se présentent dans des cas aussi définis que la calcite et l'aragonite.

Pour d'autres raisons il ne sera plus question de la forme α, ni de deux des hydrates. La forme α, très peu différente de la calcite aux points de vue géométrique et optique (Boeke), n'est connue qu'à de très hautes températures: c'est à $970° \pm 5°$ qu'elle se transforme réversiblement en calcite[1]; c'est-à-dire qu'elle n'intéresse pas le biologiste. Heide n'a d'ailleurs pas pu établir son existence aux rayons X; Smith et Adams, et aussi Eitel, qui cependant avait admis cette autonomie précédemment, la contestent formellement.

En ce qui concerne les hydrates, on ne leur connaît pas de conditions certaines de stabilité. A température assez basse ils se transforment en calcite, assez lentement pour pouvoir être étudiés. Le pentahydrate, étudié par Salm-Horstner, Scheerer, Rannelsberg, Pfeiffer, Copisarow, peut, d'après Hume, exister jusqu'à $17°$ dans une solution de sucre à 20 pour cent. D'après le même auteur il n'est pas impossible que jusqu'à $25°$, dans ces conditions, existe un hydrate inférieur, qui serait probablement le trihydrate étudié par Iwanoff. L'hexahydrate, enfin, signalé par Pelouze et depuis lors étudié par Vetter, Bütschli, Biedermann, par Johnston, Merwin et Williamson, et par Mackenzie, peut exister d'après Hume dans la solution sucrée jusqu'à $10.4°$. Mais ce sont là des conditions exceptionnellement favorables, dues à la présence de sucre: dans l'eau pure tous les hydrates sont décomposés à $0°$, et l'on ne peut les obtenir qu'au-dessous de cette température (Pelouze; Vetter; Bütschli; Biedermann; Johnston, Merwin et Williamson; Mackenzie; Hume). On peut donc s'attendre à ce qu'aucun des trois hydrates n'existe chez les êtres vivants; c'est en effet ce qui se passe. Je reparlerai, brièvement, de l'hexahydrate, parce que certains biologistes l'ont obtenu artificiellement à partir de calcaires amorphes animaux, mais les deux autres hydrates n'ont pas d'intérêt pour nous[2].

Dans ce qui suit il sera donc question de la calcite, de l'aragonite, de la vatérite, et du calcaire amorphe, qui existent chez les êtres vivants; de l'hexahydrate, qui y a été obtenu artificiellement; de la forme μ, qui peut bien, nous le verrons, avoir une existence biologique transitoire[3].

2. PROPRIÉTÉS DISTINCTIVES DES PRINCIPALES FORMES DU CALCAIRE.

Il ne peut être question d'exposer dans ce chapitre toutes les propriétés des six formes de calcaire retenues précédemment. Je me contenterai de signaler celles qui peuvent servir pratiquement, et je renverrai pour le reste aux traités de minéralogie.

$1°$. *Caractères optiques.* La calcite appartient au système rhomboédrique; c'est donc un minéral biréfringent uniaxe, donnant la croix noire en lumière polarisée convergente quand l'axe optique est parallèle à l'axe du microscope. Sa biréfringence est très forte (0.172), de sorte qu'entre nicols croisés elle donne facilement des

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[1] Le fait le plus remarquable de la transformation, selon Boeke lui-même, est un phénomène thermique.

[2] Tschirwinsky n'admet cependant que les deux hydrates $CO^3Ca, 3H^2O$, et $CO^3Ca, 5H^2O$

[3] L'existence de la forme μ, admise par Eitel, a été contestée par Spangenberg.

couleurs vives ou des irisations. Ses indices de réfraction extrêmes sont 1·658 et 1·486.

L'aragonite appartient au système monoclinique; c'est donc un minéral biaxe qui, examiné en lumière polarisée convergente, dans la direction d'une bissectrice de l'angle des axes, donne des arcs d'hyperbole au lieu de la croix noire. Ses indices de réfraction extrêmes sont sensiblement ceux de la calcite (1·686 et 1·530), et sa biréfringence est à peine plus faible (0·156), de sorte que l'on ne peut tirer de là aucun caractère différentiel pratique.

La forme μ appartient au système hexagonal; elle est donc uniaxe, comme la calcite, et son indice de réfraction moyen est le même; mais sa biréfringence est plus faible (0·1).

La vatérite, qui se présente presque toujours en sphérolithes, a été indiquée comme optiquement biaxe, mais le fait est très incertain. Selon Heide, et von Ohlshausen, qui l'ont étudiée aux rayons X, elle est du système hexagonal, donc uniaxe. Sa réfringence et sa biréfringence, plus faibles que celles de la calcite et de l'aragonite, sont quelque peu variables, l'indice de réfraction moyen s'éloignant peu de 1·55; et la biréfringence pouvant descendre à 0·13. Les sphérolithes de vatérite ont généralement un caractère optique négatif, contrairement à ceux de calcite et d'aragonite, qui sont positifs; mais ce n'est pas une différence absolue, car parfois ils sont aussi positifs.

Le carbonate amorphe, naturellement isotrope, a un indice de réfraction voisin de 1·5.

L'hexahydrate, enfin, appartient au système monoclinique, avec une biréfringence voisine de 0·085, et un indice de réfraction très bas (1·5 environ).

2°. *Caractères morphologiques.* La calcite peut se présenter en cristaux de formes diverses (rhomboèdres, prismes, scalénoèdres, isoscéloèdres, ou encore cristaux aplatis et basés), possédant tous un axe morphologique ternaire. Elle peut aussi former des sphérolithes, généralement positifs, ou encore, chez les êtres vivants, avoir des formes spiculaires, à surfaces plus ou moins courbes. Elle présente en général au moins un clivage rhomboédrique net, et souvent des macles. Quand les individus cristallins sont assez gros on peut, par les acides, y faire apparaître des figures de corrosion à symétrie ternaire (rhomboèdres et scalénoèdres), caractéristiques du système rhomboédrique.

Les formes de l'aragonite sont souvent difficiles à distinguer de celles de la calcite, mais elles n'ont pas d'axe ternaire. Il en existe aussi des sphérolithes, généralement positifs, et, chez les êtres·vivants, des formes spiculaires. Les clivages y sont incomplets, et, dans les masses fibreuses, font partie de la zone d'allongement au lieu d'être obliques comme dans la calcite. Les macles sont fréquentes. Ici encore la corrosion peut être employée pour faire apparaître des figures caractéristiques du système monoclinique.

Les cristaux artificiels de la forme μ, seuls connus actuellement, sont des plaques hexagonales, à faces planes ou marquées de six côtes rayonnantes.

La vatérite forme presque toujours des sphérolithes, généralement négatifs; on la connaît cependant aussi, semble-t-il, en petites aiguilles.

Le carbonate amorphe n'a pas de formes définies.

Le carbonate hydraté, enfin, peut se présenter en prismes ou en tablettes monocliniques.

3°. *Densité et dureté.* On connaît exactement les densités de l'aragonite et de la calcite, approximativement celles des autres formes :

aragonite : 2·95,
calcite : 2·714[1],
vatérite et μ-CO³Ca : entre 2·5 et 2·65[2],
calcaire amorphe : entre 2·25 et 2·45,
hexahydrate : 1·777 (Mackenzie).

Les duretés n'ont été déterminées que pour la calcite et l'aragonite, où elles diffèrent quelque peu : la calcite a une dureté de 3, et l'aragonite une dureté comprise entre 3·5 et 4. L'aragonite raie donc la calcite, à condition (Sorby) que l'on aille du sommet du rhomboèdre dans la direction de la diagonale inclinée ; cette condition est nécessaire pour que le critérium ait toute sa valeur ; elle a cependant rarement été observée.

4°. *Propriétés chimiques.* On a proposé un certain nombre de réactions chimiques pour distinguer aragonite et calcite.

La première en date est due à Lemberg et repose sur l'emploi du nitrate d'argent, mais les meilleures et les plus employées sont les deux réactions de Meigen. Dans l'une on agite le minéral broyé avec une solution de sulfate ferreux : l'aragonite donne ainsi un précipité abondant et vert foncé, alors que la calcite ne donne, tout au plus, qu'un précipité assez faible et jaune. Dans l'autre réaction on emploie une solution assez étendue de nitrate de cobalt : la calcite ne s'y colore pas à froid, et, même à chaud, n'y prend qu'une teinte bleu pâle ; l'aragonite s'y colore en lilas, à froid déjà, et surtout à chaud. D'après Lange il s'agirait de deux carbonates basiques de cobalt, différant par leur composition ; on pense cependant plutôt, à l'heure actuelle, que la réaction n'est pas différente, mais se fait dans les deux cas avec des vitesses différentes.

A ces réactions simples, Thugutt en a ajouté une autre que Meigen a légèrement modifiée, et qui se pratique dès lors de la façon suivante : la poudre d'aragonite, agitée une demi-minute avec une solution de nitrate d'argent centinormale, puis lavée à l'eau distillée, traitée par un excès de bichromate de potassium à saturation, et lavée à nouveau, se colore en rouge vif par formation de chromate d'argent. Dans les mêmes conditions la calcite se teint à peine. Thugutt a proposé deux autres réactions, au rouge Congo et à l'alizarine, dont la valeur est très discutée : selon lui, par ces deux colorants l'aragonite se teint en rose, alors que la calcite reste incolore.

Niederstadt, enfin, utilise pour la distinction de la calcite et de l'aragonite des réactions plus quantitatives : l'aragonite, d'après lui, est plus active dans la précipi-

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[1] Cette valeur est la valeur classique. De Foe et Compton trouvent cependant récemment, à 0°, 2·7110 ± 0·0004. Rinne trouve même 2·7101.

[2] Vater a indiqué pour la vatérite 2·536, mais Heide fait remarquer que dans ce cas les procédés ordinaires ne peuvent donner de résultats précis ; par les rayons X il évalue la densité à 2·645.

tation des sels de manganèse, de zinc et de fer; la calcite, dans la précipitation des sels de cuivre, de plomb et d'argent. Si bien que, par exemple, en ajoutant un gramme de poudre d'aragonite à une solution bouillante de sulfate de manganèse, on peut arriver à précipiter en cinq minutes 74 pour cent de la quantité théorique; dans les mêmes conditions la poudre de calcite ne précipite que 1 pour cent de la quantité théorique.

Ces diverses réactions chimiques ne doivent en aucun cas être employées avec une confiance aveugle. A peu près tous les minéralogistes sont d'accord sur ce point. Certaines d'entre ces réactions, comme celle de Thugutt et celle de Niederstadt, à l'argent, paraissent contradictoires. Bien des facteurs dont on n'est pas encore maître interviennent certainement: tels sont, selon Johnston, Merwin et Williamson, la concentration du réactif, la température, l'acidité, l'état de subdivision, et même la constitution d'ensemble du milieu solvant. Si l'on ajoute les impuretés, minérales ou autres, telles que le phosphate de calcium, les sels de fer, certains carbonates de magnésium, de baryum, de strontium, qui jouent un si grand rôle dans les calcaires animaux, et dont plusieurs minéralogistes (Meigen, Kreutz, Hutchinson, Skeats) ont signalé l'influence[1], on comprend que le contrôle de ces réactions, par d'autres procédés, soit des plus désirables.

En outre, même supposées valables pour la distinction de la calcite, les réactions chimiques en question ne peuvent permettre de différencier l'aragonite des autres formes de calcaire, et notamment de la vatérite (Heide). Ce point essentiel ne doit jamais être perdu de vue. L'essai fait par Weise pour rendre la réaction de Meigen quantitative n'a rien changé à cette imprécision.

5°. *Stabilité.* Dans les conditions normales de température et de pression la forme de calcaire stable est la calcite. Les autres, plus solubles qu'elle[2], se transforment plus ou moins rapidement en calcite lorsqu'elles sont humides. Pour le calcaire amorphe, la transformation se fait en quelques minutes; pour la vatérite, la forme μ[3] et l'hexahydrate, elle se fait en quelques heures au maximum; pour l'aragonite, au contraire, la vitesse de transformation est très faible, et le phénomène peut durer des mois et des années. Ainsi un calcaire qui, laissé au contact de l'eau, se remanie et cristallise rapidement en calcite, peut être considéré comme l'une des quatre formes précédentes. Certains types de vatérite sont cependant plus résistants, et Spangenberg a proposé à leur sujet un test, modifié par Peine, qui doit permettre de constater leur instabilité: on fait bouillir le calcaire avec de l'eau distillée pendant 15 minutes à deux jours; cet essai lui-même échoue quelquefois avec des vatérites très résistantes.

Même à l'état sec, l'aragonite et la forme μ se transforment rapidement en calcite aux températures comprises entre 400° et 500° (Sosman, Hostetter et

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[1] Il faut ajouter que, selon Panebianco, un mélange de 5 pour cent d'aragonite suffit pour qu'une calcite donne la réaction au cobalt.

[2] Des déterminations précises n'ont été faites que pour l'aragonite par Kohlrausch et Rose, Foote, Kendall, Seyler et Lloyd, Wells, Warinsky et Kouropatvinska, Bjerrum et Gjaldbaeck, Bäckström. Il en résulte que le rapport des solubilités de l'aragonite et de la calcite est à peu près 1·20. D'après Mackenzie, l'hexahydrate est à peu près deux fois plus soluble que l'aragonite et la calcite.

[3] La forme μ est instable aussi par rapport à l'aragonite, et lorsqu'elle recristallise en milieu aqueux peut donner, suivant le cas, de la calcite ou de l'aragonite.

Merwin). En ce qui concerne la vatérite, les données sont contradictoires: Johnston, Merwin et Williamson ne la trouvent pas modifiée par cette température, et ce fait leur semble indiquer que la vatérite n'est pas fondamentalement différente de la calcite; au contraire Bütschli l'avait trouvée instable, et Heide précise que la vatérite se transforme en calcite à 430° ou 440°; inchangée, à sec, jusqu'à 300° au moins, elle se transforme dès 100° en présence d'eau en calcite ou même en aragonite.

Pour le carbonate amorphe sec, la transformation en calcite se fait vers 200° déjà.

Enfin l'hexahydrate n'est peut-être stable qu'au-dessous de 0°. Au-dessus de cette température il recristallise rapidement en calcite.

6°. *Rayons X.* Le spectre des cristaux aux rayons X peut servir à déterminer les divers calcaires. Il a été établi pour la calcite par Bragg, pour l'aragonite par Wyckoff, pour la vatérite par von Ohlshausen, qui a également confirmé les observations de Bragg sur la calcite. Heide et Osawa se sont servis de ce procédé; le dernier auteur l'a même appliqué à des calcaires animaux.

En résumé les critères dont on pourra se servir pour déterminer la nature d'un calcaire animal sont les suivants.

Tout d'abord les caractères optiques, et notamment l'état biaxe ou uniaxe s'il s'agit de distinguer aragonite et calcite: ce critère exige malheureusement des cristaux assez volumineux (0·1 mm. environ) et susceptibles d'être orientés convenablement par rapport au microscope, de sorte qu'il ne peut être employé que dans certains cas.

Puis le spectre fourni par les rayons X.

Puis les caractères morphologiques, et notamment ceux tirés des figures de corrosion; ici encore, malheureusement, les masses calcaires doivent être de structure homogène sur d'assez grandes étendues, et l'emploi de ces caractères en est très restreint.

Puis la densité; en ce qui concerne les calcaires rencontrés chez les êtres vivants, l'impureté des échantillons, et notamment leur souillure par des matières organiques, font que les indications tirées de la densité sont souvent incertaines; on peut cependant assurer, entre autres choses, qu'un calcaire dont la densité dépasse 2·75 est de l'aragonite.

La dureté peut être un critérium utile, à condition de tenir compte de la direction dans laquelle on raie le cristal.

Les essais fondés sur la stabilité, assez faciles à effectuer quand il s'agit de formes très instables, sont beaucoup plus incertains en ce qui concerne l'aragonite. L'application de ce critérium n'est alors possible que dans des cas bien définis.

Les essais chimiques sont en général les plus faciles de tous à mener à bien; mais leurs résultats sont assez incertains, et ces caractères ne doivent en somme être utilisés qu'au pis-aller. Les deux réactions de Meigen suffisent d'ailleurs en général.

3. DISTRIBUTION DES DIFFÉRENTES FORMES DU CALCAIRE CHEZ LES ÊTRES VIVANTS.

Les détails qui précèdent étaient nécessaires pour donner une idée du degré de certitude avec lequel sont déterminés les divers calcaires que l'on rencontre chez les êtres vivants. Nous allons passer ceux-ci en revue, dans un ordre systématique[1]:

1⁰. *Vertébrés.*

L'os et le cartilage calcifié contiennent le calcaire sous une forme amorphe, ou tout au plus très peu biréfringente (cf. Schmidt, 1924). Le phosphate tricalcique, qui y est très abondant, peut parfois cristalliser.

Les otolithes des Vertébrés en général, et aussi les cristaux des sacs calcaires de la Grenouille, sont en aragonite (Bütschli, Kelly, Lacroix, Schmidt, d'après stabilité, indice de réfraction, densité, macles, réactions de Meigen). Il en est de même, d'après Valentin, des concrétions calcaires de l'épiphyse et des autres parties du système nerveux. D'après Kelly, cependant, les otolithes de l'Esturgeon sont en calcite (densité), et Lacroix rapporte qu'il en est de même de certaines concrétions pathologiques du rein de Bœuf.

Les coquilles des œufs d'Oiseaux sont en calcite, de même que celles de la plupart des Reptiles (Kelly, Schmidt, Meigen, d'après stabilité, propriétés optiques, densité, clivages, figures de corrosion, réactions de Meigen); celles des Tortues cependant[2] sont en aragonite (Kelly, par instabilité, indice de réfraction, clivages, densité, et réactions de Meigen; Schmidt, par examen optique et réactions de Meigen); celles des Couleuvres, enfin, sont en calcaire amorphe et instable, selon Kelly.

2⁰. *Tuniciers.*

Les spicules de la tunique des Didemnidés semblent être en aragonite (Schmidt, et moi-même, par les réactions de Meigen). Ceux des *Cystodites* sont des sphérolithes négatifs (Schmidt), caractère assez exceptionnel s'ils sont faits aussi d'aragonite. On ne connaît pas la nature minéralogique des spicules des *Pyura*, *Culeolus*, etc.

3⁰. *Mollusques.*

Les spicules de *Doris* sont des sphérolithes de vatérite (Schmidt, par densité, stabilité, propriétés optiques et réactions de Meigen). Il en est de même de ceux des *Doridae* en général, des *Pleurobranchidae*, et des concrétions calcaires du conjonctif d'autres Gastéropodes (moi-même, par stabilité et propriétés optiques). Je suis actuellement porté à considérer aussi comme une vatérite très peu biréfringente le contenu des glandes qui sécrètent l'épiphragme d'Escargot, contenu que l'on a pris pour du calcaire amorphe (Kelly, Flöszner et moi-même, par instabilité et absence de biréfringence); après son expulsion ce calcaire se transforme, et l'épiphragme est calcitique (Kelly, Schmidt, par clivages et propriétés optiques).

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[1] Pour ne pas alourdir l'exposé bibliographique, je passerai le plus souvent sous silence les travaux dont les résultats sont peu nets ou assurément controuvés. Beaucoup d'entre eux sont cités par Kelly ou par Schmidt.

[2] D'après Lacroix il en est de même de celles des Sauriens.

Parmi les coquilles de Lamellibranches, celles des Ostréides, des Pectinides et des Inocérames sont entièrement en calcite[1] (von Buch, Rose, Sorby, Kelly, Karny, Meigen, Schmidt, par densité, stabilité, clivages, caractères optiques, réactions de Meigen.) Celles des Unionides sont entièrement en aragonite (Leydolt, Rose, Sorby, von Ebner, Kelly, Biedermann, Bütschli, Karny, Schmidt, par caractères optiques, figures de corrosion, stabilité, densité, dureté, et réactions de Meigen). Dans celles des Aviculides, des Mytilides, de *Trigonia*, la couche des prismes est en calcite (les mêmes auteurs, par les mêmes caractères), alors que la nacre est en aragonite (Kelly, Neumann, Karny, Schmidt, par les mêmes caractères). Les coquilles d'Hippurites sont aussi en aragonite et calcite. Quant à celles des autres Lamellibranches, il semble qu'elles soient entièrement en aragonite à l'état adulte, mais leur texture compliquée ne permet pas de s'en assurer par les meilleurs caractères, les caractères optiques (Rose, Sorby, Kelly, Karny, Meigen, Tesch, Schmidt). D'après Kelly, les coquilles embryonnaires des *Dreissena* et des glochidies d'Anodonte sont en calcite (stabilité), alors que celles des adultes sont partiellement ou totalement en aragonite ; Schmidt observe le même fait chez la Moule (caractères optiques).

La plupart des Gastéropodes ont une coquille en aragonite (Rose, Sorby, Kelly, Bütschli, Meigen, Tesch, Schmidt, par instabilité, propriétés optiques, clivages, densité, réactions de Meigen). Il en est de même de l'opercule chez *Turbo* et *Nerita*, d'après Kelly. Chez certains Gastéropodes cependant, comme les *Scurria*, il semble qu'il y ait aussi de la calcite (Thiem, par la réaction de Meigen). Ce qui est sûr, c'est que les coquilles rudimentaires des Limaces et d'*Arion* sont en calcite, de même que celles de *Ianthina*, de *Scalaria* (Kelly, par propriétés optiques, clivages, stabilité), et de *Carinaria* (Schmidt, par propriétés optiques et réactions de Meigen). Lacroix y ajoute celles de *Cerithium* et *Melania*, et regarde celles de *Patella*, *Haliotis*, *Fusus*, comme calcitiques à l'extérieur et aragonitiques à l'intérieur[1]. Chez divers Ptéropodes, Schmidt a eu des résultats contradictoires par l'examen optique et les réactions de Meigen, mais le premier, plus digne de foi, semble bien indiquer qu'il s'agit de calcite, ce qui est d'ailleurs en contradiction avec l'opinion de Kendall.

La coquille du Dentale est faite d'aragonite (Kelly, Meigen, par indices, clivages, densité, réactions de Meigen). Les mêmes auteurs ont montré par les mêmes techniques que chez les Chitons coquille et spicules sont en aragonite. Chez les Solénogastres les caractères optiques, et aussi les réactions de Meigen, ne donnent que des résultats douteux (Schmidt).

Parmi les Céphalopodes actuels, la coquille très particulière de l'Argonaute est seule faite de calcite, alors que celles de *Sepia*, *Nautilus*, *Spirula*, sont en aragonite (Kelly, Bütschli, Meigen, par indices, clivages, stabilité, réactions de Meigen ; confirmé par Schmidt pour *Nautilus*, par l'examen optique). Les aptychus des Ammonites et les·coquilles internes des Bélemnites étaient calcitiques, alors que la coquille des Ammonites était aragonitique (cf. Lacroix).

Le tube de *Teredo* est en calcite, tandis que ceux de *Gastrochaena* et d'*Aspergillum* sont en aragonite (Kelly, par densité, indices et stabilité). Le dard de l'Escargot

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[1] Lacroix cite cependant les Spondyles et Inocérames comme présentant une nacre aragonitique.

est aussi en aragonite (Kelly, par stabilité). Le statolithe de *Pterotrachea* est en calcite (Schmidt). Enfin les coques des œufs des *Helix, Ampullaria, Bulimus, Amphidromus,* sont en calcite (Turpin, Rose, Kelly, Schmidt, par clivages, indices, réactions de Meigen).

4°. *Arthropodes.*

Les téguments calcifiés des Cirripèdes sont en calcite (Kelly, Bütschli, Meigen, par densité, propriétés optiques, clivages, stabilité, réaction de Meigen). Il en est de même pour un grand nombre d'Ostracodes (Kelly, Schmidt, par propriétés optiques et stabilité), et de Décapodes, Brachyures et Anomoures surtout (Kelly, Schmidt, moi-même, par propriétés optiques et stabilité). Chez d'autres Crustacés, ou même dans certaines parties des précédents, le calcaire est amorphe (Kelly, Bütschli, Biedermann, Schmidt, moi-même); c'est le cas surtout chez la plupart des Macroures, beaucoup d'Amphipodes, certains Isopodes, *Nebalia,* certains Ostracodes.... Chez d'autres, enfin, appartenant aux mêmes groupes, le calcaire amorphe est mêlé à de la calcite ou à de la vatérite (propriétés optiques, instabilité). Partout où existe le calcaire amorphe, l'action de l'eau peut le transformer rapidement, à froid en hexahydrate, et à la température ordinaire en calcite (Bütschli, Biedermann, moi-même).

Dans le tégument du Myriapode *Iulus* aussi, Kelly et Bütschli ont reconnu du calcaire amorphe et instable, tout-à-fait semblable à celui des Crustacés.

Les "yeux d'Écrevisse," de l'estomac de cet animal, sont en calcite (Kelly, par clivages).

Les concrétions sphérolithiques du tissu adipeux de certains Insectes (larves de Phytomyzines et d'Anthomyzines) sont en vatérite (moi-même, par caractères optiques et instabilité).

5°. *Vers et groupes voisins.*

Dans la première paire de glandes de Morren, chez les Lombrics, le calcaire est à l'état de calcite (Kelly, Schmidt, par densité et caractères morphologiques), tandis que dans la seconde il est à l'état amorphe et instable (Kelly, Schmidt). Peut-être, dans ce dernier cas, sa forme en globules indique-t-elle, à mon avis, plutôt une vatérite à très faible biréfringence.

Les tubes calcaires des Serpuliens ont été considérés par Bütschli et par Meigen comme formés de calcite, et par Kelly comme faits de "conchite," c'est-à-dire d'aragonite. La question reste pendante à l'heure actuelle.

Les concrétions sphérolithiques du parenchyme des Cestodes sont en vatérite (moi-même, par caractères optiques et instabilité). Il en est probablement de même chez certains Trématodes, où l'on en a signalé d'analogues.

Chez les Bryozoaires calcaires il s'agit de calcite (Kelly, Meigen, par densité, clivages, propriétés optiques, réactions de Meigen); Sorby, cependant, avait cru, probablement à tort, à l'aragonite.

Chez les Brachiopodes à coquille calcaire (Brachiopodes Articulés, et *Crania*), le calcaire est aussi de la calcite (Sorby, Kelly, Bütschli, Meigen, Schmidt, d'après

les propriétés optiques, les clivages et la densité). Il en est de même des spicules du conjonctif chez les mêmes Brachiopodes (Schmidt, d'après les propriétés optiques).

6°. *Échinodermes.*

Il est bien connu que dans ce groupe les plaques calcaires et les spicules sont toujours faits de calcite (Hessel, Leydolt, Bütschli, von Ebner, Kelly, Meigen, Becher, Merker, Schmidt). Nulle part peut-être le fait n'est mieux établi, par plus de procédés convergents.

7°. *Cœlentérés.*

Chez les Octocoralliaires en général les spicules sont faits de calcite. Les *Heliopora*, qui sont quelque peu aberrants à d'autres égards, forment au contraire de l'aragonite, de même que les Hexacoralliaires et les Hydrocoralliaires. Ces faits sont bien établis par les propriétés optiques, les densités, la stabilité, les clivages et les réactions de Meigen (Sorby, Kelly, Meigen, Schmidt).

8°. *Spongiaires.*

Les spicules calcaires des Éponges en général sont en calcite, comme il résulte notamment des études optiques et autres très poussées de Sollas, von Ebner, Bidder.

Cependant les spicules aberrants et sphérolithiques des Astrosclérides sont en aragonite (Hutchinson, Schmidt, par densité et réactions de Meigen). Selon Kirkpatrick, d'ailleurs, il s'agit là, non pas de vrais spicules, mais de cellules d'Algues calcifiées.

9°. *Foraminifères.*

Les Foraminifères qui ont été étudiés à ce point de vue sont tous calcitiques (Sollas, Sorby, von Ebner, Kelly, Meigen, Schmidt, par les procédés optiques, la stabilité, les clivages, les réactions de Meigen).

10°. *Végétaux.*

Parmi les Algues calcaires, certaines, comme les *Halimeda, Acetabularia, Galaxaura, Cymopolia*, sont en aragonite, alors que les *Lithothamnium, Lithophyllum, Melobesia*, sont en calcite.

Je n'ai trouvé aucune indication bibliographique sur la nature du calcaire d'autres Végétaux, en particulier sur celle des cystolithes.

En résumé :

On trouve du calcaire amorphe dans les glandes de Morren postérieures des Lombrics, dans la coque des œufs de Couleuvre, et surtout dans les téguments de nombreux Arthropodes ; il faut y ajouter la matière minérale des os de Vertébrés, qui n'est, il est vrai, calcaire que pour une faible part.

On trouve de la vatérite dans certaines concrétions du conjonctif chez les Mollusques, les Cestodes, probablement quelques Trématodes, et dans le tissu adipeux de quelques Diptères.

L'aragonite est plus fréquente, et caractérise: la plupart des otolithes des Vertébrés, la coque des œufs de Tortues, les spicules des Didemnidés, la coquille de la plupart des Mollusques, les tubes des *Gastrochaena* et *Aspergillum*, le dard de l'Escargot, le squelette des *Heliopora*, des Hexacoralliaires et des Hydrocoralliaires, celui des Astrosclérides, et enfin certaines Algues calcaires.

Sauf quelques cas indécis (tubes de Serpule, spicules des Aplacophores, cystolithes), tous les autres calcaires semblent être de la calcite.

L'hexahydrate n'est jamais qu'un produit artificiel. La forme μ, connue d'ailleurs depuis peu, n'a jamais été rencontrée chez les êtres vivants.

4. LES DONNÉES ACTUELLES SUR LES CAUSES QUI DÉTERMINENT LA PRODUCTION DES DIFFÉRENTES FORMES DE CALCAIRE.

1º. *La production d'aragonite.*

Dès que le dimorphisme du carbonate de calcium fut connu, il y a plus d'un siècle, on se préoccupa de ses causes. Depuis lors on a beaucoup écrit sur elles, sans que, d'ailleurs, les travaux récents aient modifié de façon essentielle et définitive les résultats acquis il y a plus de cinquante ans.

Le fait fondamental et unanimement reconnu à l'heure actuelle est que la calcite est la forme stable du carbonate de calcium dans les conditions normales de température et de pression. Dans ces conditions toutes les autres formes tendent à se transformer en calcite, plus ou moins rapidement, alors que la transformation inverse est impossible[1]. La vitesse de transformation, grande pour le carbonate amorphe ou la vatérite, est très faible au contraire pour l'aragonite. Cette différence de stabilité va naturellement de pair avec une différence de solubilité dans l'eau: la calcite, forme la plus stable, est naturellement la moins soluble.

Il résulte de là, en apparence, que la production de calcite, du moins lorsqu'elle est directe, n'a pas besoin d'être expliquée. Les efforts ont donc porté sur l'explication des formes moins stables, et notamment de l'aragonite, qui est à peu près la seule envisagée. J'examinerai successivement les différentes hypothèses, dans l'ordre chronologique où elles ont été proposées.

1º. *Influence des carbonates isomorphes de l'aragonite.* Haüy, le premier, considéra que l'aragonite ne peut se produire que si elle renferme du strontium, et admit que toutes les aragonites en renferment nécessairement. Stromeyer fit sienne cette hypothèse, et signala dans les aragonites typiques de Vertaizon et de Bastennes des teneurs en strontiane de 1·45 pour cent et 2·88 pour cent. Credner porta la question sur le terrain expérimental, et fit voir que lorsque du bicarbonate de calcium se dissocie en présence de quantités suffisantes de bicarbonate de strontium, le précipité formé est de l'aragonite, dans laquelle le strontium peut être décelé au spectroscope. Il fit voir, de plus, que le carbonate de plomb a le même effet que le carbonate de strontium. Bauer ayant étendu ces résultats au carbonate de baryum, on réunit tous ces faits en considérant que les carbonates isomorphes de

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[1] On admet assez généralement que l'aragonite doit avoir une zone de stabilité vers 100°, et une autre aux hautes pressions, mais ces conditions ne peuvent évidemment intervenir dans les êtres vivants.

l'aragonite peuvent entraîner la cristallisation à l'état d'aragonite, à condition de se précipiter avant celle-ci, c'est-à-dire à condition que le rapport de leur métal au calcium soit assez élevé.

On devrait s'attendre, dans ces conditions, à ce qu'une cristallisation de calcaire, effectuée en présence d'aragonite, donnât de l'aragonite. Les expériences de Vetter, puis celles de Johnston, Merwin et Williamson, prouvent qu'il n'en est rien, et que les germes cristallins isomorphes de l'aragonite ne sont efficaces qu'à l'état naissant. La seule présomption indirecte du contraire est fournie par une observation de Cullis : dans l'atoll de Funafuti les vides des Coraux aragonitiques qui viennent de mourir se remplissent d'aragonite ; lorsque cependant ces vides sont revêtus de vase qui isole l'aragonite préexistante, il se fait de la calcite.

L'influence, sur la formation d'aragonite, des germes cristallins isomorphes naissants, est unanimement admise à l'heure actuelle. Inversement (Vetter ; Johnston, Merwin et Williamson) des germes de calcite empêchent la formation d'aragonite dans des conditions de température qui devraient déterminer celle-ci.

Mais, depuis Stromeyer, personne n'a prétendu que dans le carbonate de strontium, ou même dans les carbonates isomorphes, fût l'explication unique de la production d'aragonite. Credner, Vogelsang, Bauer, et aussi Johnston, Merwin et Williamson sont plus éclectiques. Un fait est certain, en tous cas : il y a des aragonites sans strontium, contrairement à ce que pensait Haüy ; Rose l'a affirmé ; Leitmeier en cite des exemples, en partie d'après Des Cloizeaux ; après Vauquelin et Laugier, qui avaient sérieusement réduit la teneur en strontium des aragonites étudiées par Stromeyer, Lacroix n'a trouvé que 1·20 pour cent de strontiane dans celle de Bastennes, 0·38 pour cent dans celle de Dax ; il n'en a pas trouvé du tout dans celle de Framont, et n'a pu en déceler, même au spectroscope et par les procédés les plus sensibles, dans l'aragonite des marnes sparnaciennes d'Issy ; de même, Warynski et Kouropatwinska n'ont trouvé au spectroscope que des traces de strontium dans une aragonite. Inversement il existe des strontianocalcites renfermant plusieurs centièmes de carbonate de strontium, des plombocalcites à 9 pour cent de carbonate de plomb, et Vater a obtenu expérimentalement des calcites contenant 16 pour cent de carbonate de baryum. On voit que la teneur en carbonates isomorphes de l'aragonite n'est pas forcément décisive.

En ce qui concerne les calcaires animaux nous possédons peu de renseignements. Seul Vogel signale au spectroscope du strontium chez des Coraux et des Lamellibranches ; certains de ces animaux sont en aragonite, mais il trouve, en outre, du strontium chez des Huîtres, calcitiques. Rien de net ne peut être tiré pour le problème actuel de ces observations insuffisantes.

On doit conclure, en somme, que les carbonates isomorphes de l'aragonite peuvent avoir une influence sur la formation de celle-ci, mais que cette influence ne peut être exclusive.

2°. *Influence de la température.* Rose, le premier, invoqua l'effet de la température. Alors qu'à la température ordinaire la précipitation du calcaire donne de la calcite, celle-ci se trouve mêlée d'aragonite dès la température de 30° ; à 70° l'aragonite prédomine, et se forme seule à 90°. Ces faits ont été très généralement

confirmés, au moins en partie, par les auteurs plus récents: Harting, Vogelsang, Vater, Meigen, Linck, Hatschek, Vetter, Leitmeier. Tous, y compris Rose, sont cependant d'accord pour admettre que l'influence de la température n'est pas la seule à agir, et que d'autres peuvent interférer avec elle. Il est clair, d'ailleurs, en ce qui concerne les calcaires des êtres vivants, que la température n'a rien à voir avec leurs formes cristallines.

Il faut noter que récemment Johnston, Merwin et Williamson, tout en reconnaissant le sens général du phénomène, refusent d'admettre que l'aragonite se forme dès 30°. Ce que l'on a pris pour l'aragonite, à cette température, est, selon eux, la forme μ. La calcite se ferait seule au-dessous de 30°; puis apparaîtrait la forme μ, et enfin l'aragonite apparaîtrait aussi au-dessus de 50°. Cette correction ne change pas le sens du phénomène.

En aucun cas on ne doit interpréter, comme l'a fait Linck, la formation d'aragonite comme la preuve de sa stabilité au voisinage de 100°. Il s'agit simplement d'une accélération de la précipitation, qui permet aux molécules de se grouper suivant un autre type que le plus stable. Vetter et Leitmeier invoquent à ce propos la loi d'Ostwald, suivant laquelle doit se faire d'abord une forme métastable, qui plus tard se transforme lentement en la forme stable.

3°. *Influence de la concentration.* C'est Rose aussi qui signala l'importance de la concentration: à la température ordinaire, selon lui, on obtient de l'aragonite par les solutions diluées, de la calcite par les solutions plus concentrées, l'effet étant si net que la production d'aragonite peut succéder à celle de calcite. Admis par Vogelsang et par Kelly, et décrit à nouveau par Credner dans la précipitation de solutions de bicarbonate par départ du gaz carbonique, le fait a été énergiquement nié par Vater; cet auteur a été jusqu'à considérer que Credner avait pris des Bactéries pour des cristaux d'aragonite; quant à Rose, il n'aurait pas suffisamment caractérisé son aragonite. Johnston, Merwin et Williamson n'admettent pas non plus l'influence de la concentration.

4°. *Influence de l'équilibre des carbonates.* La question n'a jamais été posée sous la forme précise que je lui donne. Mais chez quelques auteurs on peut trouver des préoccupations de cet ordre. Harting, par exemple, croit à l'influence du gaz carbonique libre, qui favoriserait la précipitation de la calcite; Rose, confirmé par Adler, précise que dans ces conditions on obtient de la calcite, même à haute température. Vogelsang observe: "Harting a déjà fait remarquer que la forme du vase n'est pas indifférente, et j'ai trouvé aussi que les mêmes substances, mêlées dans les mêmes proportions à la même température, donnent des cristallites dans les tubes à essai, et des rhomboèdres dans les capsules plates." Vater nie formellement toute influence de cet ordre.

Je signalerai ici l'opinion de Warth et de Meigen, suivant qui la production d'aragonite est favorisée par la réaction alcaline du milieu; évidemment erronée, cette opinion repose sur une détermination insuffisante de l'aragonite, sans doute confondue, par les moyens chimiques, avec la vatérite ou le calcaire amorphe.

5°. *Influence du sulfate de calcium.* Dans une ancienne expérience, Becquerel avait obtenu de l'aragonite par précipitation du bicarbonate de sodium par une

plaque de gypse. Credner montra plus tard que le sulfate de calcium déterminait la formation d'aragonite. Revu, dans certains cas du moins, par Linck, le fait ne l'a été ni par Cornu ni par Vetter. Vater l'a nié énergiquement: pour lui le sulfate de calcium, comme d'ailleurs tous les sels concomitants, est capable de déformer les cristaux de calcite, mais non pas de changer le système cristallin; outre ses expériences il s'appuie sur les observations de Pöhlmann et de Doss, suivant lesquelles de la calcite peut se faire en présence de gypse ou d'eaux séléniteuses. Johnston, Merwin et Williamson semblent cependant avoir remis en évidence le fait que le sulfate de calcium (seul parmi tous les sels qu'ils ont employés) favorise la formation d'aragonite: ils l'interprètent en admettant qu'il fait avec l'aragonite une solution solide moins soluble et par conséquent plus stable que la calcite; la teneur critique en sulfate de calcium serait, selon eux, 1 pour cent environ.

Peut-on invoquer, en ce qui concerne les calcaires animaux ou végétaux, l'influence du sulfate de calcium? Pour en juger nous disposons d'analyses assez nombreuses, dues à Clarke et Wheeler ou rapportées par eux.

CALCITES.

Spongiaires: 2 analyses de Clarke et Wheeler, 1 de Bütschli: o à 1·8 pour cent.

Alcyonaires: 21 analyses de Clarke et Wheeler, 2 de Bütschli: traces à 5·43 pour cent. Dans 19 de ces analyses, la teneur est supérieure à 1 pour cent.

Échinodermes: 60 analyses de Clarke et Wheeler, 2 de Bütschli, 4 de Schmelck: traces à 3·56 pour cent. En général, il est vrai, la teneur est assez basse.

Bryozoaires: 10 analyses de Clarke et Wheeler: de 1·32 à 8·47 pour cent.

Brachiopodes calcaires: 1 analyse de Schmelck, 3 de Kunckell, 5 de Clarke et Wheeler: o·36 à 2·15 pour cent.

Ostraea: 10 analyses de Chatin et Müntz donnent de o·779 à 1·004 d'acide sulfurique, pour 100 de matière sèche, ce qui, calculé en sulfate de calcium et rapporté à la calcite seule, fait à peu près 1 à 1·3 pour cent.

Argonauta: 1 analyse de Bütschli donne 1·46 pour cent.

Cirripèdes: 1 analyse de Balane, par Bütschli, donne 1·80 pour cent.

Algues: 19 analyses de Mélobésiées, par Clarke et Wheeler: de o à 1·39 pour cent.

ARAGONITES.

Madréporaires: 3 analyses de Clarke et Wheeler: o à o·21 pour cent.

Heliopora: 1 analyse de Clarke et Wheeler: o·50 pour cent.

Hydrocoralliaires: 1 analyse de Lenox, 6 de Clarke et Wheeler: o·06 à 2·08 pour cent.

Amphineures: 1 analyse d'un Chiton, par Clarke et Wheeler: o·35 pour cent.

Gastropodes: 3 analyses, par Clarke et Wheeler: o à o·2 pour cent.

Céphalopodes: 3 analyses, par Bütschli: o·17 à o·69 pour cent.

Algues: 2 analyses de Damour, et 4 de Clarke et Wheeler, portant sur *Halimeda* et *Galaxaura*: o·05 à 2·32 pour cent.

En somme, alors que la teneur des calcites en sulfate de calcium varie de o à 8·47 pour cent, celle des aragonites va de o à 2·32 pour cent seulement. En moyenne, celle des aragonites est plutôt inférieure; le fait est même assez net dans certains groupes, comme les Céphalopodes et les Alcyonaires. En tous cas rien de général ne peut être tiré des tableaux précédents. Il ne semble donc pas que le sulfate de calcium intervienne de façon très efficace dans la production d'aragonite chez les êtres vivants. Doss, au surplus, avait déjà montré que de la calcite contenant

4 pour cent de sulfate de calcium pouvait se former dans la nature aux dépens d'eaux séléniteuses. On peut conclure que si le sulfate de calcium a un effet sur la production d'aragonite, cet effet n'est ni suffisant, ni nécessaire.

6°. *Influence des sels de magnésium*. Linck, Cornu, Vetter, Hlawatsch, Leitmeier ont montré que la présence, dans les eaux-mères, de sulfate ou de chlorure de magnésium en quantité suffisante, déterminait la formation d'aragonite. Vater, puis Johnston, Merwin et Williamson l'ont expressément nié.

Chez les êtres vivants, en tous cas, nous allons voir que les sels de magnésium ne jouent pas ce rôle déterminant. Il est clair, en effet, que là où nous trouvons plus de magnésium dans le calcaire la teneur en sels de magnésium dans les eaux-mères était particulièrement forte. Les analyses chimiques des calcaires nous donnent donc un moyen de nous faire une idée de cette teneur en magnésium. Or leurs résultats, notés tout d'abord par Bütschli, puis par Biedermann, et par Clarke et Wheeler, sont très nettement défavorables aux vues de Linck et de ses successeurs: l'aragonite est toujours pauvre en sels de magnésium; la calcite en contient ordinairement beaucoup, bien qu'il y ait des exceptions dans des groupes bien définis. Une revue rapide va le montrer (les teneurs données sont calculées en $MgCO^3$).

CALCITES.

Foraminifères: 7 analyses de Clarke et Wheeler, plusieurs autres rapportées par Brady: en général 8·8 à 12·52 pour cent (chez trois espèces cependant: 4·5; 3·67 et 1·79 pour cent).

Spongiaires: 4 analyses de Clarke et Wheeler, 1 de Bütschli: 4·61 à 14·10 pour cent.

Alcyonaires: 21 analyses de Clarke et Wheeler, 1 de Anderson (rapportée par Murray et Renard), 2 de Bütschli, 13 de Phillips (rapportées par Clarke et Wheeler): 6 à 16·90 pour cent. D'autres analyses moins utilisables, dues à divers auteurs, et rapportées par Clarke et Wheeler, donnent aussi des teneurs élevées.

Échinodermes: 98 analyses de Bütschli, de Schmelck, et surtout de Clarke et Wheeler, ou de Palmer, rapportées par Clarke et Wheeler: 4·8 à 14·75 pour cent.

Bryozoaires[1]: 2 analyses de Schwager (rapportées par Walther), 13 de Clarke et Wheeler: 0·17 à 11·08 pour cent.

Brachiopodes calcaires: 5 analyses de Clarke et Wheeler, 1 de Sharples, 2 de Künckell, 2 de Schmelck: traces à 1·40 pour cent chez les Articulés; 3·5 à 8·63 pour cent chez *Crania*.

Pectinidés et Ostréidés: 3 analyses de Clarke et Wheeler, 10 de Chatin et Müntz: 0·70 à 1 pour cent.

Argonauta: 1 analyse de Bütschli, 1 de Clarke et Wheeler: 5·37 et 6·02 pour cent.

Cirripèdes[2]: 1 analyse de Bütschli, 7 de Clarke et Wheeler, 1 de moi-même: 0·75 à 2·49 pour cent.

Algues calcitiques: 3 analyses de Damour, 2 de Schwager (rapportées par Walther), 1 de Gümbel, 11 de Högbom, 7 de Mme Lemoine, 1 de Nichols, 3 de Châlon, 1 de Skeats, 20 de Clarke et Wheeler: 3·76 à 25·17 pour cent.

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[1] Clarke et Wheeler rapportent et discutent, à propos des Hydrocoralliaires, une donnée de Damour, relative à "*Millepora cervicornis*." D'après la localité indiquée (Bréhat), il y a tout lieu de croire qu'il ne s'agit pas d'un fossile, comme le croient ces auteurs, mais d'une Mélobésiée, ou plutôt encore du Bryozoaire *Eschara cervicornis*. Rien d'étonnant, dès lors, à ce qu'il ait 8·51 pour cent de $MgCO^3$.

[2] Chez les autres Crustacés, où le calcaire est, soit de la calcite, soit du calcaire amorphe ou de la vatérite, la teneur en carbonate de magnésium est encore plus élevée, d'après les analyses de Clarke et Wheeler, et les miennes propres, qui seront données ailleurs. Il faut seulement excepter les Crustacés d'eau douce, où la teneur en magnésium est très basse, comme l'a vu aussi Bütschli.

ARAGONITES.

Hexacoralliaires: 30 analyses de Clarke et Wheeler; diverses autres analyses de Sharples et de Lenox, rapportées par Clarke et Wheeler: 0·09 à 1·11 pour cent.

Heliopora: 1 analyse de Clarke et Wheeler: 0·35 pour cent.

Hydrocoralliaires[1]: 1 analyse de Lenox, 6 de Clarke et Wheeler: 0·22 à 2·14 pour cent; d'autres analyses, de Högbom et Sharples, donnent des valeurs inférieures à 1 pour cent.

Lamellibranches aragonitiques: 8 analyses de Clarke et Wheeler: 0 à 0·46 pour cent.

Dentalium: 1 analyse de Clarke et Wheeler: 0·20 pour cent.

Chiton: 1 analyse de Clarke et Wheeler: 0·45 pour cent.

Gastéropodes probablement aragonitiques: 33 analyses de Clarke et Wheeler: en général de 0 à 0·51 pour cent; 2 exceptions atteignant 1·78 pour cent.

Céphalopodes: 3 analyses de Bütschli, 2 de Clarke et Wheeler: 0·16 à 1·62 pour cent.

Algues aragonitiques: 2 analyses de Damour, 4 de Clarke et Wheeler, 1 de Högbom: traces à 1·04 pour cent.

L'opposition entre la plupart des calcites et les aragonites est très nette. Alors que dans les aragonites[2] la teneur en carbonate de magnésium varie de 0 à 2·14 pour cent, dans les calcites elle ne tombe pas au-dessous de 3·5 pour cent dans de nombreux groupes, Brachiopodes articulés, Cirripèdes et Lamellibranches mis à part (ainsi que quelques Foraminifères et Bryozoaires exceptionnels); elle peut s'élever à 25·17 pour cent. Les faits sont d'autant plus remarquables que la teneur en magnésium dépend de la température du milieu, comme l'ont fait ressortir Clarke et Wheeler: ce facteur étranger n'arrive pas à troubler la loi.

Les faits sont plus nets encore dans des groupes délimités: parmi les Alcyonaires, *Heliopora* seul, qui est en aragonite, a une teneur de 0·35 pour cent, alors que tous les autres, qui sont en calcite, varient de 6 à 16·90 pour cent; parmi les Céphalopodes, l'Argonaute, qui est le seul calcitique, atteint 5 à 6 pour cent, alors que les autres, qui sont en aragonite, ne dépassent pas 1·62 pour cent; parmi les Algues, les *Halimeda* et *Galaxaura*, aragonitiques, ne dépassent pas 1·04 pour cent, alors que les Mélobésiées, calcitiques, varient de 3·76 à 25·17 pour cent.

Même parmi les Mollusques (sauf les Céphalopodes), où les teneurs sont bien plus faibles, Bütschli a remarqué depuis longtemps que la teneur de 0·5 pour cent en carbonate de magnésium semblait être une teneur critique. Les listes de Clarke et Wheeler semblent, il est vrai, apporter deux exceptions à ce principe, parmi les Gastéropodes, mais l'ensemble des faits n'en est pas moins fort net. On doit faire ressortir, en particulier, l'opposition entre les Pectinidés et Ostréidés calcitiques, et les autres Lamellibranches.

Cependant de l'examen d'ensemble ne ressort en aucune façon, à moins que d'autres conditions interviennent dans certains cas, un effet déterminant des sels de magnésium: si ces derniers agissaient seuls, les Lamellibranches calcitiques, par exemple, devraient être en aragonite, tout comme certains Hexacoralliaires plus riches qu'eux-mêmes en magnésium. Au surplus, je le répète, l'action des sels magnésiens serait inverse de celle que feraient prévoir les indications de Linck et de ses successeurs.

[1] Nous avons déjà vu ce qu'il faut penser du "*Millepora*" analysé par Damour.

[2] Encore faut-il, d'après Clarke et Wheeler, admettre, pour les sels magnésiens de l'eau de mer imprégnant les échantillons analysés, une correction qui peut atteindre 0·4 pour cent, et qui réduit en général à 0 la teneur vraie en carbonate de magnésium.

7°. *Influence des sels d'ammonium.* Adler, Meigen, Peine ont indiqué que la présence de sels d'ammonium ou d'ammoniaque libre favorisait la formation d'aragonite. Vater, Vetter, et aussi Johnston, Merwin et Williamson n'ont pas retrouvé ce résultat[1]. Il est dû, probablement, à des déterminations insuffisamment précises de l'aragonite.

8°. *Influences diverses.* Bien d'autres sels ont été essayés, sans succès, par Vater, Cornu, Vetter, et Johnston, Merwin et Williamson; ces derniers ont en particulier étudié à ce point de vue les divers sels de l'eau de mer. Vater a posé en principe, à la suite de longues études, que les sels concomitants ("*Lösungsgenossen*") peuvent modifier la forme géométrique de la calcite, mais sont impuissants à changer le système cristallin du calcaire. Seul Leitmeier[2] trouve que, outre le chlorure de magnésium, d'autres sels facilement dissociables peuvent déterminer la formation d'aragonite, et le peuvent à une concentration d'autant plus faible que la température est plus élevée; pour les concentrations insuffisantes on n'influe que sur la forme géométrique des cristaux de calcite, comme le dit Vater.

Bourgeois ayant obtenu un mélange de calcite et d'aragonite en chauffant à 140° un sel de calcium en présence d'urée, Vater croit à une influence de l'urée, favorisante pour l'aragonite, et rappelle que dans l'urine de Lapin le calcaire se présente à l'état d'aragonite.

Vogelsang invoque, à côté d'autres conditions, le rapport quantitatif des solutions qui réagissent pour amener la précipitation. Meigen admet, de même, que dans les précipitations de chlorure de calcium par le carbonate de sodium un excès de chlorure empêche en général la formation d'aragonite. Il s'agit ici, au fond, d'une question d'alcalinité, que nous avons déjà envisagée.

A propos de ces influences diverses je signalerai que si, profitant des données des analyses, nous comparons dans les aragonites et les calcites les teneurs en phosphate de calcium, en silice, en oxydes de fer et d'aluminium, rien de net ne ressort de ces comparaisons. Tout au plus peut-on constater, dans certaines calcites, un taux de ces diverses impuretés plus variable que dans les aragonites, et atteignant parfois des valeurs plus élevées; mais ce n'est en rien un fait général.

En résumé on peut conclure, avec Johnston, Merwin et Williamson, que trois faits sont établis *in vitro*:

1°. La production d'aragonite est favorisée par la présence de germes cristallins isomorphes naissants.

2°. Elle est favorisée par la présence de sulfate de calcium en quantité suffisante.

3°. Elle est d'autant plus facile que la température est plus voisine de 100°[3].

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[1] Miron et Bruneau, précédemment, avaient d'ailleurs obtenu de la calcite par action d'air chargé d'ammoniaque sur de l'eau de rivière.

[2] Linck admet cependant aussi que dans les solutions salines l'aragonite est moins soluble (donc plus stable) que dans l'eau pure, et que le cas est inverse pour la calcite. Il est probablement erroné, ici encore, d'admettre que l'aragonite se forme parce qu'elle est stable.

[3] A ces trois conditions les auteurs ajoutent la production d'aragonite par les êtres vivants: c'est le problème qui nous est posé.

A ces faits ajoutons la constatation que les aragonites, biologiques du moins, ne contiennent pour ainsi dire jamais de magnésium, alors que les calcites sont souvent très riches en ce métal, et nous aurons fait le bilan complet des résultats. Beaucoup des autres vues émises sont controuvées. Certaines autres, à peine esquissées par leurs auteurs, seront reprises de façon plus précise dans le prochain chapitre.

2°. *La production des formes instables, autres que l'aragonite.*

Nous sommes encore bien moins renseignés sur l'origine des autres formes instables, et nous n'avons guère ici que des recettes de production.

1°. *Forme μ.* Johnston, Merwin et Williamson obtiennent cette forme, d'ailleurs mêlée de calcite et d'aragonite, à des températures comprises entre 30° et 60°.

2°. *Vatérite.* Vater l'a obtenue pour la première fois par précipitation en présence de sels de baryum.

Bütschli, Spangenberg, Heide l'ont faite, avec des tours de main légèrement différents, par cristallisation secondaire lente d'un gel de calcaire précipité.

Vetter dit l'avoir obtenue plusieurs fois par dissociation du bicarbonate de calcium dans l'eau de mer; mais, à considérer les procès-verbaux d'expériences, il ne paraît pas maître de sa production. Pour lui, c'est le magnésium de l'eau de mer qui favoriserait la production de la vatérite, comme celle des autres formes instables. Vetter dit aussi produire de la vatérite en accélérant par l'ammoniaque le départ de gaz carbonique.

Johnston, Merwin et Williamson obtiennent la vatérite par précipitation en milieu alcalinisé par la potasse.

3°. *Calcaire amorphe.* Bien que le calcaire amorphe se produise très fréquemment dans les précipitations, et peut-être pour cette raison, on ne trouve aucun renseignement précis sur les conditions où il se fait. Tout au plus sait-on que la concentration des solutions réagissantes doit être assez élevée. Je ne reviendrai pas ici sur les tours de main par lesquels Bütschli, et aussi Neuberg et Rewald, l'ont obtenu à l'état sec.

4°. *Hexahydrate.* Il est bien établi (Pelouze; Vetter; Bütschli; Biedermann; Johnston, Merwin et Williamson; Mackenzie) que l'hexahydrate ne se fait qu'à basse température, et généralement au-dessous de 0°; il peut atteindre 10° dans une solution sucrée. A l'effet favorisant de la basse température et des matières organiques, Vetter ajoute celui de la sursaturation en bicarbonate, de la présence de carbonate d'ammonium, et de l'absence de formes plus stables.

5. CONCLUSION, ET ESQUISSE D'UN PLAN DE TRAVAIL.

Au terme de cette revue des travaux publiés, nous arrivons donc à une conclusion décevante: si les minéralogistes et les chimistes, travaillant *in vitro* sur la production d'aragonite, peuvent compter à leur actif, d'une part l'influence de la température, d'autre part celle des minéraux isomorphes, et peut-être aussi celle du sulfate de

calcium, les biologistes, à qui les conditions de formation sont données et, en grande partie tout au moins, différentes de celles-là, ne peuvent se satisfaire de ces résultats. Les températures supérieures à 50° ne sauraient se rencontrer chez les êtres vivants ; les faits, nous l'avons vu, ne justifient pas le rôle que l'on voudrait attribuer au sulfate de calcium ; et l'effet des minéraux isomorphes ne peut être rendu responsable dans tous les cas.

Et cependant, plus encore que les minéralogistes, les biologistes doivent compter sur un déterminisme parfait des formes minéralogiques du calcaire. Lorsque Johnston, Merwin et Williamson, *in vitro*, échouent à déterminer à volonté aragonite ou calcite par les trois conditions qu'ils reconnaissent importantes, ils invoquent en quelque sorte le hasard, et plus précisément la chute, dans les solutions réagissantes, de poussières calcaires qui servent de noyaux de cristallisation, soit pour l'aragonite, soit pour la calcite. Le biologiste ne peut laisser une telle part au hasard ; il sait que dans l'ensemble des conditions que résume le chimisme d'un tissu donné d'un animal donné, il n'y a pas place pour le hasard, puisque le résultat est toujours le même ; il sait, en particulier, que l'introduction accidentelle de cristaux déterminants est impossible dans les cas qui l'intéressent ; il sait même, par l'exemple de la coquille des Aviculides et des Mytilides, qu'une cristallisation d'aragonite peut succéder régulièrement à une cristallisation de calcite, ce qui peut sembler un double paradoxe.

Aussi est-il obligé d'admettre que les conditions posées jusqu'à présent, et en particulier celles de Johnston, Merwin et Williamson, ne sont pas nécessaires, et qu'il doit y en avoir d'autres, différentes, et peut-être plus importantes, puisqu'elles peuvent triompher, et de la présence de sulfate de calcium, pour faire apparaître de la calcite, et de celle de cristaux de calcite, pour faire apparaître de l'aragonite.

J'ai entrepris de rechercher ces conditions de façon méthodique. Il est frappant en effet de constater que les chimistes qui ont étudié cette question ont opéré à l'aventure, en quelque sorte, sans préciser suffisamment les conditions des expériences, et parfois (Meigen, Vetter par exemple) dans un désordre presque inextricable. On peut préciser les choses très exactement en prenant pour base les conditions d'équilibre des carbonates en solution.

Le début du calcul de cet équilibre a été esquissé par Clark ; je le développerai ailleurs avec ses conséquences. On obtient, pour une température donnée, entre les trois variables (concentration en ions Ca, pression partielle du gaz carbonique P, et concentration en ions hydrogène), l'équation :

$$[Ca] = \frac{108 \cdot 10^{-20} P + 9 \cdot 10^{-9} [H] P + 10^{-14} [H] - [H]^3}{2 [H]^2}.$$

En donnant différentes valeurs à P on peut tracer un réseau d'abaques correspondant aux différentes valeurs de la pression de gaz carbonique. Il est commode pour cela, d'opérer avec une échelle logarithmique, et de porter en abscisses les pH, et en ordonnées les logarithmes des concentrations en ions Ca. En modifiant légèrement le calcul on peut tracer un second réseau, où la variable correspondant au gaz

carbonique est, non plus la pression partielle, mais la quantité totale C de gaz carbonique, combiné ou non, de la phase liquide. L'équation en est:

$$[Ca] = C \frac{36.10^{-18} + 3.10^{-7}[H]}{2\,[18.10^{-18} + 3.10^{-7}\,[H]] + [H]^2} + \frac{10^{-14} - [H]^2}{2\,[H]}.$$

Pour les faibles concentrations en calcium, ces courbes coïncident avec les courbes correspondantes établies en fonction des pressions, mais elles s'en détachent, par suite de l'effet tampon, pour les concentrations élevées de calcium, et tendent alors asymptotiquement vers la courbe limite, correspondant à une pression nulle de gaz carbonique.

A ce double réseau, représenté dans la figure 1, on peut associer, les coordonnées restant les mêmes, les courbes de solubilité de la calcite, de la chaux, du bicarbonate de calcium, dont les équations traduisent respectivement en fonction de [H] et de [Ca], les conditions:

$$[CO^3]\,[Ca] = k,$$
$$[Ca]\,[OH]^2 = k',$$
$$[Ca]\,[CO^3H]^2 = k'',$$

où k, k' et k'' sont des constantes.

En portant log [Mg] en ordonnées à la place de log [Ca], on peut y associer aussi les courbes de solubilité, tout-à-fait semblables aux précédentes, du carbonate de magnésium, de la magnésie et du carbonate de magnésium. Si l'on voulait tracer, en outre, les courbes de solubilité des calcaires instables, elles seraient très voisines de celle de la calcite, mais situées un peu au-dessous d'elle.

L'ensemble de ces courbes est donné dans la figure 1. Obtenu par le calcul, il a besoin d'être vérifié expérimentalement. C'est ce que j'ai fait pour un assez grand nombre de points, et toujours le résultat de l'expérience a été d'accord avec celui du calcul. Ce réseau de courbes permet de résoudre théoriquement les problèmes de précipitation des calcaires, et même des calcaires magnésiens.

La courbe de solubilité de la calcite partage, tout d'abord, le plan en deux régions. La région inférieure, I, correspond à des solutions non-saturées en calcium. La précipitation du calcaire ne peut s'y faire que par départ de gaz carbonique, et se fait, en principe, en équilibre. Elle ne peut donner, en ce cas, les formes très instables, dont la courbe de solubilité est entièrement et largement au-dessus de celle de la calcite, puisque cette dernière est rencontrée tout d'abord par le point figuratif de l'équilibre, en cas de départ de gaz carbonique[1]. L'expérience prouve cependant que ce premier mode de précipitation peut donner calcite ou aragonite, puisque les dépôts d'eaux souterraines, qui en relèvent, peuvent être formés de l'un ou l'autre de ces minéraux. Comme les mesures de solubilité de l'aragonite ont été faites aussi bien sous la tension normale du gaz carbonique dans l'atmosphère, c'est-à-dire à un pH de 8·5 environ, que (Foote) dans de l'eau préalablement saturée de gaz carbonique sous une atmosphère, c'est-à-dire à un pH voisin de 6, et que dans tous les cas la solubilité était supérieure à celle de la calcite, on est forcé

[1] Johnston, Merwin et Williamson remarquent déjà que les formes instables ne peuvent se faire que s'il y a solution sursaturée par rapport à leur solubilité.

d'admettre que dans tout la région pratique du plan, au moins, l'aragonite est réellement instable. Pour qu'elle se produise il faut donc qu'il se fasse une sursaturation par rapport à la calcite, et la question ne peut plus être discutée théoriquement, mais doit être étudiée par l'expérience. Là interviennent peut-être des germes cristallins isomorphes; ou peut-être les conditions spéciales d'évasion du gaz carbonique amènent-elles plus ou moins facilement la sursaturation nécessaire.
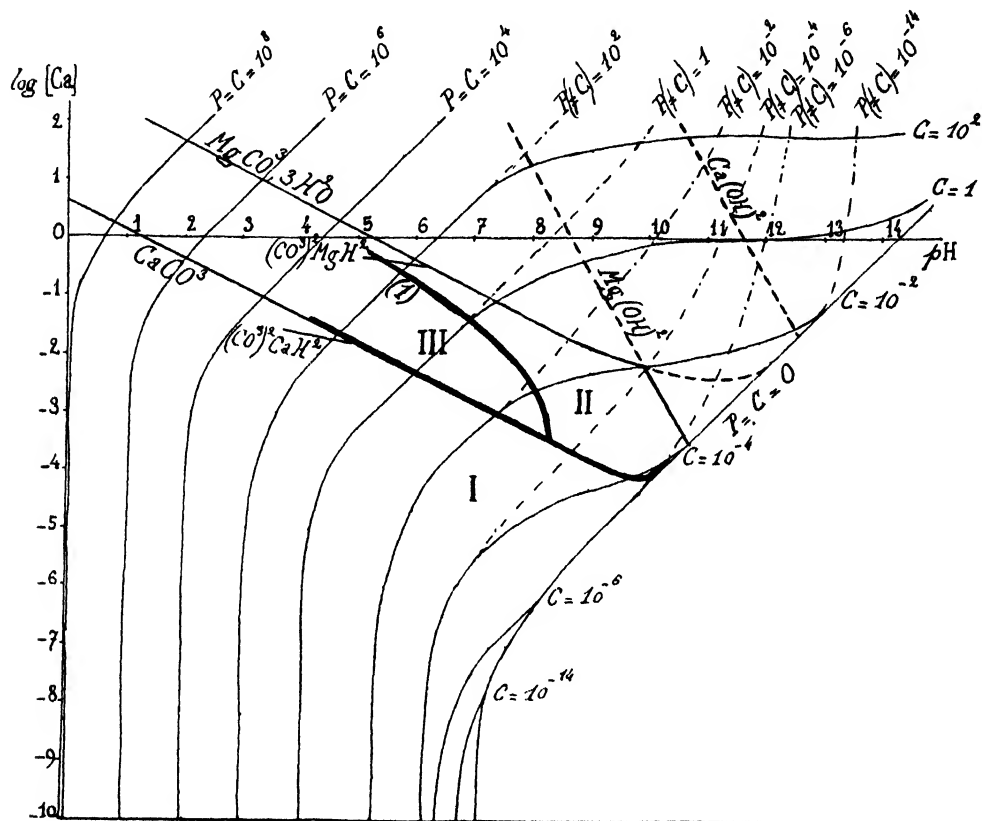

Fig. 1. Diagramme de l'équilibre des carbonates, en fonction de la pression du gaz carbonique P, de sa concentration C, du pH et du logarithme de la concentration en ions métal. On y a porté, en outre, les courbes de solubilité de plusieurs composés considérés, et la courbe 1, lieu géométrique des points où, au cours de la précipitation, la quantité de gaz carbonique dégagé est moléculairement égale à la quantité de calcaire précipité.

A cette région I du plan se rattachent, au moins partiellement, les travaux de Rose, Credner, Vater, Linck, Vetter. Ceux de Vetter sont particulièrement suggestifs s'il est exact qu'il ait pu, en accélérant le départ de gaz carbonique, obtenir même de la vatérite, forme encore plus instable que l'aragonite. Cette question est à reprendre, et l'on n'oubliera pas qu'à ce mode de précipitation, où l'équilibre est peut-être légèrement modifié par la présence de matières organiques, se rattache la formation des coquilles chez les Mollusques, les Brachiopodes, vraisemblablement les Cirripèdes, et aussi, probablement, celle des spicules de Didemnidés.

L'autre région du plan correspond à un équilibre instable, pratiquement réalisé par la précipitation d'un carbonate alcalin par un sel soluble de calcium, comme dans une partie des expériences de Rose, Credner, Vater, Cornu, Linck, Meigen, et de Johnston, Merwin et Williamson. Le point figuratif au départ de la précipitation est toujours facile à déterminer par le pH et la tension de gaz carbonique. La précipitation elle-même ne se fait pas en équilibre, et son allure dépend des conditions extérieures, en particulier de la forme du récipient, qui rend plus ou moins facile l'évasion du gaz carbonique : on retrouve ici une idée de Harting et de Vogelsang.

Expérimentalement la précipitation se fait suivant deux modes différents, qui correspondent à deux subdivisions très nettes de cette région. Dans la plus alcaline (région II) la précipitation est immédiate et aboutit à du calcaire amorphe, métastable, et voué à une cristallisation plus ou moins rapide[1]. Dans la plus acide (région III) la précipitation est retardée, et d'emblée cristalline. La ligne de démarcation entre ces deux régions, telle que la donne l'expérience, coïncide remarquablement avec une courbe théorique. Le calcul montre en effet que lorsqu'une certaine quantité de calcium sort de la phase liquide il en sort toujours en même temps une quantité de gaz carbonique moléculairement plus grande. La différence entre ces deux quantités est faible pour les hauts pH et les fortes concentrations en calcium ; elle est grande pour les bas pH et les faibles concentrations en calcium. Cette différence correspond à un dégagement de gaz carbonique. Or si l'on calcule le lieu géométrique des points pour lesquels ce dégagement est moléculairement égal au calcaire précipité, on constate qu'il coïncide avec la ligne de démarcation entre les régions II et III. Autrement dit, la région III correspond à des solutions provisoirement sursaturées en bicarbonate de calcium, et la région II à celles où le carbonate précipite d'emblée. Dans ce dernier cas, le précipité est tout d'abord amorphe. Dans l'autre lorsqu'il se fait, avec un certain retard, il est immédiatement cristallin.

Le calcaire amorphe pur cristallise rapidement, en quelques instants pour les pH bas, en une heure au plus pour les pH élevés. Il existe cependant, relativement stable, dans les téguments de certains Crustacés. J'ai pu constater que cette stabilité étonnante est due à ce qu'il est impur, et mêlé d'une forte proportion de phosphate tricalcique. Quand la cristallisation se fait à bas pH et à faible concentration elle donne de la calcite ; à pH élevé et à forte concentration, elle produit de la vatérite, qui elle-même, si les conditions changent ensuite, peut se transformer en calcite. Ces deux types de cristallisation se rencontrent aussi dans les téguments de certains Crustacés, malgré une certaine stabilisation du calcaire amorphe, due au mélange de phosphate tricalcique[2]. Mais surtout c'est à eux que se rattachent d'une part les vatérites intracellulaires (conjonctif des Gastéropodes, Cestodes, Diptères), et d'autre part bon nombre de calcites d'origine intracellulaire également (Échinodermes, Spongiaires, probablement Alcyonaires et spicules des Brachiopodes) ;

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[1] C'est évidemment dans ces conditions qu'opéraient Rose, Foote, Copisarow, Divers, Bütschli.

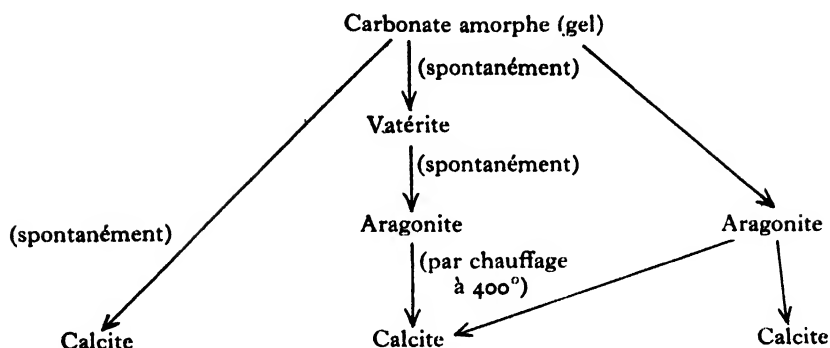[2] Toute cette question sera développée ailleurs.

ceci justifie donc, mais en partie seulement, l'idée de Kelly, que la calcite est toujours secondaire, et succède au calcaire amorphe[1].

L'étude de la région III n'est pas terminée; on y rencontre en effet d'assez sérieuses difficultés dans la détermination même des minéraux obtenus, étant données les faibles dimensions des précipités. Il est certain que dans cette région peuvent se faire de l'aragonite et de la calcite. C'est là aussi, très probablement, que se produit la forme μ de Johnston, Merwin et Williamson, et il n'est pas impossible que celle-ci soit la forme métastable, vite transformée, suivant les conditions, soit en calcite, soit en aragonite. Mais tout ceci exige encore quelques recherches. Cette région paraît d'ailleurs moins intéressante au point de vue biologique: il n'est même pas sûr qu'aucun calcaire animal ou végétal se forme dans ces conditions.

Cette systématisation d'ensemble, il est facile de la voir, englobe et coordonne presque tous les faits épars connus. On savait que le carbonate amorphe se formait en milieu relativement alcalin et concentré, et que la vatérite exigeait une forte alcalinité. L'action de la température, qui favorise la formation d'aragonite, s'en déduit aussi probablement: les abaques ont été tracées aux environs de la température ordinaire; à température plus élevée elles sont modifiées par un triple phénomène (diminution de solubilité du gaz carbonique, augmentation de la dissociation de l'eau, augmentation de la dissociation de l'acide carbonique), de sorte qu'en fin de compte elles sont toutes déplacées vers la gauche, vers la région où peut se former l'aragonite. De même encore l'influence des sulfates, que Johnston, Merwin et Williamson attribuent à la formation d'une solution solide, pourrait bien être due plutôt à ce que l'introduction de sulfates dans une solution de carbonates déforme les courbes d'équilibre et les déplace vers la gauche, comme le calcul le prévoit et comme l'expérience le confirme. Enfin le fait que les calcites seules peuvent renfermer du carbonate de magnésium s'explique très aisément par le simple examen de la courbe de solubilité de ce dernier: aux tensions de gaz carbonique pratiques, tout au moins, elle n'a pas de rapports avec la région III du plan, où peut se faire l'aragonite. Seule échappe à notre systématisation l'influence des minéraux isomorphes, influence bien connue par ailleurs et d'un autre ordre.
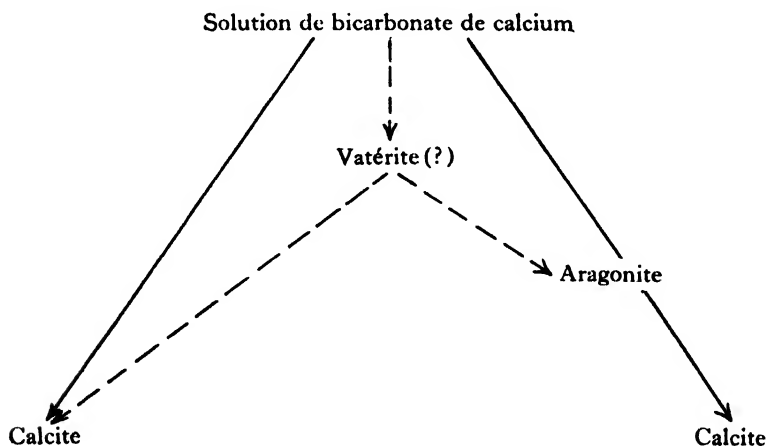
A vrai dire seule peut être considérée comme suffisamment avancée l'étude de la région II du plan, une des plus importantes d'ailleurs au point de vue biologique. Pour les autres nous avons un plan de travail, plutôt que des résultats positifs précis. Mais les résultats négatifs sont déjà fort importants: il ne peut se faire, quelles que soient les causes accessoires que l'on fait agir, ni aragonite dans la région II du plan, ni calcaire amorphe et vatérite dans la région III. On conçoit dès lors qu'il y ait eu contradiction entre des auteurs qui opéraient en quelque sorte au hasard. On conçoit aussi l'inexactitude du schéma suivant donné par Linck pour les relations génétiques entre les différentes sortes de calcaires.

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[1] Kelly a émis de plus, de façon très dubitative, l'idée que l'absence de formation du calcaire amorphe était le facteur déterminant de l'apparition de "conchite," c'est-à-dire d'aragonite. Foote a repris cette opinion avec plus de force, en l'appuyant sur le principe d'Ostwald.
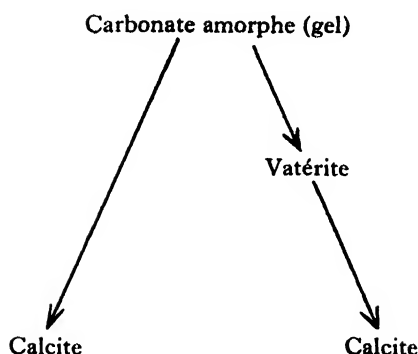
Carbonate amorphe (gel)

(spontanément)

Vatérite

(spontanément)

(spontanément)   Aragonite   Aragonite

(par chauffage
à 400°)

Calcite   Calcite   Calcite

Il est faux que le carbonate amorphe soit l'origine de tous les calcaires; il est faux que dans des conditions simples[1] il puisse donner de l'aragonite; il est faux que l'aragonite soit un stade intermédiaire nécessaire entre la vatérite et la calcite, si même, dans des conditions simples, elle peut être un intermédiaire entre elles. Ainsi, au schéma unique de Linck nous devons substituer trois schémas indépendants, correspondant aux trois régions du plan.
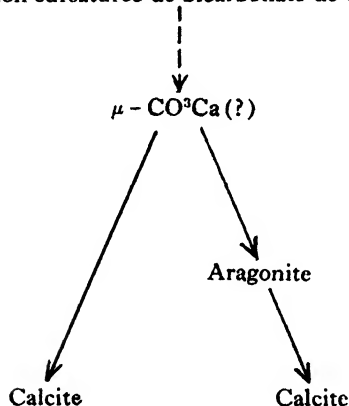
Région I:

Solution de bicarbonate de calcium

Vatérite (?)

Aragonite

Calcite   Calcite

Région II:

Carbonate amorphe (gel)

Vatérite

Calcite   Calcite

Région III:

Solution sursaturée de bicarbonate de calcium

$\mu - CO^3Ca$ (?)

Aragonite

Calcite   Calcite

Les principales incertitudes qui subsistent ressortent clairement de ces schémas eux-mêmes. Il faut encore une œuvre de longue haleine pour les dissiper et pour préciser les causes accessoires qui, dans le premier et le troisième cas, orientent la cristallisation dans telle ou telle des voies possibles.

INDEX BIBLIOGRAPHIQUE.

ADLER, M. (1897). "Beiträge zur Kenntnis des kohlensauren Kalkes." *Zeitschr. f. angew. Chem.*

BÄCKSTRÖM, H. L. J. (1921). "Ueber die Affinität der Aragonit-Calcit Umwandlung." *Zeitschr. f. physik. Chemie,* **97.**

BAUER (1890). "Beiträge zur Mineralogie. XII. Ueber eine Pseudomorphose von Aragonit nach Kalkspath." *Neues Jahrb. Miner.*

BECHER, S. (1914). "Ueber die Benutzung des Polarisationsmikroskops zur morphologischen Analyse des Echinodermenskelettes." *Zool. Jahrb., Anat.* **38.**

BECQUEREL (1831). "Du carbonate de chaux cristallisé, et de l'action simultanée des matières sucrées ou mucilagineuses sur quelques oxydes métalliques par l'intermédiaire des alcalis et des terres." *Ann. Chim. Phys.* [2], **47.**

BIDDER (1898). "The skeleton and calcification of calcareous Sponges." *Proc. Roy. Soc. London,* **64.**

BIEDERMANN (1901). "Ueber den Zustand des Kalkes im Crustaceenpanzer." *Biol. Centralbl.* **21.**

BJERRUM, N. et GJALDBAECK, T. K. (1919). *D. Kgl. Veterinär- og Landbohöjskole Aarsskrift.* Kopenhagen.

BOEKE, H. E. (1912). "Die Schmelzerscheinungen und die umkehrbare Umwandlung des Calciumcarbonates." *Neues Jahrb. Miner.*

—— (1915). *Grundlagen der physikalisch-chemischen Petrographie.*

BOURGEOIS, L. (1886). "Nouveaux procédés de préparation des carbonates cristallins." *C. R. Ac. Sc.* **103**.

BRAUNS, R. (1901). "Ueber das Verhältnis von Conchit zu Aragonit." *Centralbl. Miner. Geol.*

BUCH, von (1828). "Ueber Silicifikation organischer Körper." *Abh. der Berliner Akad.*

BÜTSCHLI, O. (1908). "Untersuchungen über organische Kalkgebilde, nebst Bemerkungen über organische Kieselgebilde." *Abh. d. kgl. Ges. d. Wiss. Göttingen, math.-phys. Kl.*, N.F. **6**.

CHATIN, A. et MÜNTZ, A. (1895). "Analyse des coquilles des Huîtres." *C. R. Ac. Sc.* **120**.

CLARK, W. M. (1922). *The determination of hydrogen ions.* Baltimore.

CLARKE, F. W. et WHEELER, W. C. (1922). "The inorganic constituents of marine Invertebrates." *U.S. Geol. Survey*, Prof. paper 124.

COPISAROW, M. (1923). "Heteromorphism of calcium carbonate. Marble, synthetic and metamorphic." *Journ. Chem. Soc. London*, **123**.

CORNU (1907). *Oesterr. Z. Berg. Hüttenwesen*, **55**.

CREDNER, H. (1870). "Ueber gewisse Ursachen der Krystallverschiedenheiten des kohlensauren Kalkes." *Journ. f. prakt. Chem. 2.*

CULLIS, C. G. (1904). *The atoll of Funafuti.* XIV. The mineralogical changes.

DAMOUR, A. (1851). *C. R. Ac. Sc.* **32**.

DIVERS, E. (1870). "On the precipitation of solutions of ammonium carbonate, sodium carbonate, and ammonium carbonate by calcium chloride." *Journ. Chem. Soc. London*, **8**.

DOSS, B. (1897). "Ueber livländische durch Ausscheidung aus Gypsquellen entstandene Süszwasserkalke, als neue Beispiele für 'Mischungsanomalien.'" *Neues Jahrb. Miner.*

EBNER, von (1887). "Ueber den feineren Bau der Kalkschwämme, nebst Bemerkungen über Kalkskelette überhaupt." *Sitzber. d. Wiener Akad., Math.-naturw. Kl.* **95**.

EITEL, W. (1923). Nouvelle édition de: Boeke, *Grundlagen der physikalisch-chemischen Petrographie.*

—— (1924). *Neues Jahrb. f. Miner.*

FOE, O. K. de, et COMPTON, A. H. (1922). *Phys. Rev.* (2), **25**.

FOOTE, H. W. (1900). "Ueber die physikalisch-chemischen Beziehungen zwischen Aragonit und Calcit." *Zeitschr. physik. Chemie*, **33**.

GÜMBEL, C. W. (1871). *K. Bayer. Akad. Wiss. Abh., Math.-phys. Kl.* **11**.

HARTING, P. (1840). "Étude microscopique des précipités et de leurs métamorphoses." *Bull. Soc. Phys. et Nat. de Néerlande.*

HATSCHEK, E. (1909). "Die Krystallform des aus konzentrierten Lösungen gefällten Carbonats." *Chem. Zeitschr.* **33**.

HEIDE, F. (1924). "Ueber den Vaterit." *Centralbl. f. Miner. u. Geol.*

—— (1925). "Nachtrag zu der Mitteilung über die 'Vaterit' genannte Modification des CaCO³." *Centralbl. f. Miner. u. Geol.*

HESSEL (1826). *Einflusz des organischen Körpers auf den inorganischen.* Marburg, 1826.

HLAWATSCH, C. (1909). "Der Aragonit von Rohitsch." *Zeitschr. f. Krystall.* **47**.

HÖGBOM, A. G. (1894). "Ueber Dolomitbildung und dolomitische Kalkorganismen." *Neues Jahrb. f. Miner.*

HUME, J. (1925). "The hydrates of calcium carbonate." *Journ. Chem. Soc. London*, **127**.

HUTCHINSON, A. (1902). "On the mineralogical character of the skeleton of Astrosclera." *Willey's Zoolog. results, Cambridge.*

IWANOFF (1908). "Ein wasserhaltiges Calciumcarbonat aus der Umgebung von Nowo Alexandria." *Zeitschr. f. Krystall. u. Miner.* **44**.

JOHNSTON, J., MERWIN, H. E. et WILLIAMSON, E. D. (1916). "The several forms of calcium carbonate." *Amer. Journ. of Sc.* (4), **41**.

KARNY, H. (1913). "Optische Untersuchungen zur Aufklärung der Struktur der Muschelschalen." *Sitzber. Akad. Wiss. Wien, Math.-naturw. Kl.* **122**.

KELLY, A. (1901). "Beiträge zur mineralogischen Kenntnis der Kalkausscheidungen im Tierreich." *Jen. Zeitschr.* **35**.

KENDALL (1896). "On the cause of the bathymetric limit of Pteropod ooze." *Rep. Brit. Assoc.* 1896.

KOHLRAUSCH et ROSE (1893). "Die Löslichkeit einiger schwer löslichen Salze im Wasser." *Zeitschr. physik. Chemie*, **12**.

KOSSMANN, K. (1892). "Hydrocalcit, ein neues Calciumhydrocarbonat." *Zeitschr. d. deutsch. geol. Gesellsch.* **44**.

KREUTZ, S. (1909). "Ueber die Reaktion von Meigen." *Miner. petr. Mitth.* **28**.

KUNCKELL, F. (1899). "Ueber die chemische Zusammensetzung der Schalen von Crania, Terebratulina und Waldheimia." *Journ. prakt. Chemie* (2), **59**.

LACROIX (1898). "Sur la ctypéite, nouvelle forme de carbonate de calcium, différente de la calcite et de l'aragonite." *C. R. Ac. Sc.* **126.**
—— *Traité de minéralogie.*
LANGE (1904). *Inaug.-Diss. Freiburg.*
LEITMEIER, H. (1909). "Die Absätze des Mineralwassers von Rohitsch-Sauerbrunn in Steiermark." *Zeitschr. f. Krystall.* **47.**
—— (1911). "Calcit, Aragonit." *Doelter's Handbuch der Mineralchemie,* **1.**
LEMBERG, J. (1892). "Zur mikrochemischen Untersuchung einiger Minerale." *Zeitschr. d. deutsch. geol. Gesellsch.* **44.**
LEMOINE, Mme (1911). "Structure anatomique des Mélobésiées." *Ann. Inst. Océan. Monaco,* **2.**
LENOX, L. R. (1904). *Harvard Coll. Mus. Comp. Zool. Bull.* **44.**
LEYDOLT (1856). "Ueber die Struktur und Zusammensetzung der Krystalle des prismatischen Kalkhaloïds nebst Anhang über die Skelette der kalkigen Teile einiger wirbellosen Tiere." *Sitzber. der Wiener Akad.* **19.**
LINCK, G. (1903). "Die Bildung der Oolithe und Rogensteine." *Neues Jahrb. Miner.,* Beil. Bd. **16.**
—— (1909). "Ueber die Bildung der Kalksteine." *Naturw. Wochensch.* **8.**
MACKENZIE, J. E. (1923). "Calcium carbonate hexahydrate." *Journ. Chem. Soc. London,* **123.**
MEIGEN, W. (1901). "Eine einfache Reaktion zur Unterscheidung von Aragonit und Kalkspath." *Centralbl. Miner. Geol.*
—— (1902). "Die Unterscheidung von Kalkspath und Aragonit auf chemischem Wege." *Ber. des Oberrhein. Geol. Ver.* **35.**
—— (1903 et 1905). "Beiträge zur Kenntnis des kohlensauren Kalkes." *Naturf. Gesells. Freiburg Ber.* **13** et **15.**
MERKER, E. (1916). "Studien am Skelett der Echinodermen." *Zool. Jahrb. Physiol.* **36.**
MIRON et BRUNEAU (1882). "Reproduction de la calcite et de la withérite." *C. R. Ac. Sc.* **90.**
MURRAY, J. et RENARD, A. F. (1891). "Deep sea deposits." *Challenger Report.*
NEUBERG et REWALD (1908). *Bioch. Zeitschr.* **9.**
NICHOLS, H. W. (1906). *Field Columbian Mus. Publ.* III.
NIEDERSTADT (1912). *Zeitschr. f. angew. Chem.* **25.**
OHLSHAUSEN, S. von (1925). "Strukturuntersuchungen nach der Debye-Scherrer Methode." *Zeitschr. f. Krystall.* **61.**
OSAWA, A. (1925). *Science reports Tohohu Imp. Univ.* (I), **14.**
PANEBIANCO, G. (1902). *Rivista Min. Crist. ital.* **28.**
PEINE, J. (1913). *Inaug.-Diss. Jena.*
PELOUZE (1831). "Sur la production artificielle du carbonate de chaux cristallisé, et sur deux combinaisons de ce sel avec l'eau." *Ann. Chim. Phys.* (2), **48.**
POHLMANN, R. (1892). "Mineralogische Mittheilungen." *Verh. d. deutsch. wissensch. Ver. zu Santiago,* **2.**
PRENANT, M. (1924). "Contributions à l'étude cytologique du calcaire. 1. Quelques formations calcaires du conjonctif chez les Gastéropodes." *Bull. Biol. Fr. Belge,* **58.**
QUERCIGH, E. (1916). *Rivista Min. Crist. ital.* **44.**
RINNE, F. (1923). "Ermittlung des specifischen Gewichtes von Steinsalz und Kalkspat auf röntgenometrischem Wege." *Centralbl. Miner. u. Geol.*
—— (1924). "Röntgenographische Untersuchungen an einigen feinzerteilten Mineralien, Kunstprodukten und dichten Gesteinen." *Zeitschr. f. Krystall.* **60.**
ROSE, G. (1856). "Ueber die heteromorphen Zustände der kohlensauren Kalkerde." *Abh. der Berliner Akad.*
SALM-HORSTNER, W. F. zu (1835). "Untersuchung eines krystallinischen Kalksalzes." *Ann. Phys. u. Chem.* **35.**
SCHEERER (1846). *Ann. Phys. u. Chem.* **68.**
SCHMELCK, L. (1901). *Norske Nordhaws Exped.* No. 28.
SCHMIDT, W. J. (1924). *Die Bausteine des Tierkörpers im polarisierten Lichte.* Bonn, 1924.
SEYLER et LLOYD (1909). "Studies of the carbonates. I. The equilibrium between calcium carbonate and carbonic acid." *Journ. Chem. Soc. London,* **95.**
SHARPLES, S. P. (1871). *Amer. Journ. Sci.* (3), **1.**
SKEATS, E. W. (1904). "The Atoll of Funafuti." *London Royal Soc. Proc.*
SMITH, F. H. et ADAMS, L. H. (1923). *Journ. Amer. Chem. Soc.* **45.**
SOLLAS (1885). "Physical characters of calcareous and siliceous sponge spicules." *Sci. Proc. Roy. Dublin Soc.* **4.**
SORBY, H. C. (1879). "Presidential address." *Quart. Journ. Geol. Soc.* **35.**
SOSMAN, HOSTETTER et MERWIN (1915). *Journ. Wash. Acad.* **5.**
SPANGENBERG, K. (1913). "Die künstliche Darstellung des Dolomits." *Zeitschr. f. Krystall.* **52.**
STROMEYER (1813). "De aragonite ejusque differentia a spathe calcareo rhomboidali chemica." *Comment. Soc. Reg. scient. Gotting.* **2.**

Tesch, P. (1908). *Verslag v. d. Vergad. d. Wiss. Nat. Afd. kgl. Akad. Amsterdam.*

Thiem (1917). "Beiträge zur Anatomie und Phylogenie der Docoglossen." *Jen. Zeitschr.* **54.**

Thugutt (1910). "Ueber chromatische Reaktionen auf Calcit und Aragonit." *Centralbl. Miner., Geol.*

Tschirwinsky, P. (1911). "Die Hydrate des Calciumcarbonats." *Doelter's Handb. der Mineralchemie,* **1.**

Vater, H. (1893). "Ueber den Einflusz der Lösungsgenossen auf die Krystallisation des Calciumcarbonates. I." *Zeitschr. f. Krystall. u. Miner.* **21.**

—— (1894). "*Id.* II. Krystallisation des Calciumcarbonates aus sogenannten verdünnten Lösungen." *Ibid.* **22.**

—— (1895). "*Id.* III. Die Beeinflussung der Homogenität und der Wachstumsgeschwindigkeit der Kalkspathkrystalle durch dilut färbende Substanzen." *Ibid.* **24.**

—— (1895). "*Id.* IV. Die von Gustav Rose dargestellten und als Aragonit beschriebenen garbenförmigen und dergleichen Aggregate sind durch den Einflusz färbender Substanzen zerfaserte Kalkspathkrystalle." *Ibid.* **24.**

—— (1897). "*Id.* V. Die scheibenförmigen Krystalliten des Calciumcarbonates." *Ibid.* **27.**

—— (1899). "*Id.* VI. Schwellenwerth und Höhenwerth der Lösungsgenossen bei ihrem Einflusse auf die Krystallisation." *Ibid.* **30.**

—— (1899). "*Id.* VII. Der Einflusz des Calciumsulfates, Kaliumsulfates und Natriumsulfates." *Ibid.* **30.**

—— (1899). "*Id.* VIII. Ueber die Einwirkung von Alkalicarbonatlösungen auf Gyps und Anhydrit." *Ibid.* **31.**

—— (1902). "Ueber Ktypeit und Conchit." *Zeitschr. f. Krystall.* **35.**

Vetter, F. (1911). "Beiträge zur Kenntnis der Ausscheidungen des kohlensauren Kalkes aus Bicarbonatlösungen." *Zeitschr. f. Krystall.* **48.**

Vogel, O. (1894). "Ueber die Anwendung der Leuchtgassauerstoffflamme zu spektralanalytischen Mineraluntersuchungen." *Zeitschr. anorg. Chemie,* **5.**

Vogelsang, H. (1875). *Die Krystalliten.* Bonn, 1875.

Walther, J. (1885). *Deutsch. Geol. Gesellsch. Zeitschr.* **37.**

Warth, H. (1902). "Die Bildung des Aragonits aus wässriger Lösung." *Centralbl. f. Miner., Geol.*

Warynsky, T. et Kouropatwinska, S. (1916). "Étude sur l'équilibre isotherme du système CO^3Ca cristallisé + NH^4Cl aq." *Journ. Chim. Phys.* **14.**

Weise, C. (1923). "Ueber sphaerolithische Calcium-Magnesium Carbonate." *Dissert. Jena.*

Wells, R. C. (1920). *Journ. Wash. Acad. Sci.* **10.**

Wyckoff, R. W. G. (1925). *Amer. Journ. Sci.* (5), **9.**

ABSTRACTS OF COMMUNICATIONS
MADE TO THE SOCIETY

February 28th, 1927

NOTES ON PERIODIC PHENOMENA OF PLANT LIFE IN MALAYA
By R. E. Holttum

DETAILED series of observations on the periodic phenomena of plant life in the moist tropics have hitherto been made principally in Ceylon and in Java. A recent paper of Coster's[1] gives the results of observations in East Java, with a general discussion of the matter and full reference to previous work. Broadly speaking, it has been found that in the majority of plants showing a marked periodicity in leaf-fall, the production of new leaves, and flowering, these phenomena bear a definite relation to climatic changes (the incidence of dry and wet seasons) though there are many cases, notably of species which produce new leaves and flowers at frequent intervals, which have appeared to be independent of climatic conditions. These have been considered to be governed by an internal rhythm rather than by external conditions, the latter being always sufficiently favourable to allow of their life processes being carried on. Two things have struck me in relation to published observations concerning this question: first, that the localities in which the observations have been made all show a quite definite dry or notably drier season, whereas in the south of the Malay Peninsula no such regular dry season exists; second, that in comparing the behaviour of trees from year to year, often no correlation has been attempted between changes in this and climatic differences from year to year. It has therefore appeared to me that Singapore is a more suitable place for such investigations than Ceylon or any part of Java, and that what is required is a series of observations over several years, correlated with meteorological records.

During my residence in Singapore from 1922 to 1926, though not primarily interested in this matter, I have been struck by the differences of climate from year to year, and with the fact that many plants seem to react to these differences. Though on an average no month in the year in Singapore has less than 6 inches of rain (the driest months being February and June) there is much variation (*e.g.* February had 1·71 ins. in 1923 and 17·16 ins. in 1924) and occasionally there may be a rather pronounced dry period, especially about February, following the wet and cloudy months of November to January. On the other hand, there is normally a marked dry period, though not usually quite rainless, in Penang and the north of the Peninsula from about January to March. The only published observations of the behaviour of plants in the Peninsula are those by Dr F. W. Foxworthy, who has lately collected information concerning dates of flowering and fruiting of timber trees in various parts of the Peninsula, some of which are published in the Annual Report of the Conservator of Forests, F.M.S., for the year 1925. They indicate that in some part of the area individual species have been found flowering and fruiting in almost every month of the year.

It appears that Penang has a climate, as regards rainfall, roughly similar to that of Peradaniya, and so far as I have observed the trees there behave much as recorded by Wright for the latter locality. During the dry period at the beginning of the year, deciduous trees lose their leaves; a few, such as *Erythrina indica*, flower while bare of leaves, while others flower when the new leaves appear. There is generally a great burst of flowering, especially during the later part of the dry season, of an intensity never seen in Singapore. For example, a species of Bougainvillaea flowers freely which in Singapore will only produce a few flowers at long intervals. There is a heavy flowering of fruit trees at this time, followed by a fruit crop later in the year.

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[1] "Lauberneuerung und andere periodische Lebensprozesse in dem trockenen Monsungebiet Ost-Java's." *Ann. Buitenzorg,* **33** (1923), 117–89.

When a dry period occurs in the early part of the year in Singapore the same kinds of phenomena are observed as at Penang, but usually in less degree, as there is rarely so marked a drought as in the north. All the Hevea trees lose their leaves; there is a heavy general flowering of both native and exotic plants, especially noticeable in introduced shrubs which hardly flower at other times. Fruit trees flower and produce a crop of fruit later. There are other years when instead of a dry period heavy rain continues throughout February, or at least the month is moderately rainy. In these years the leaf-fall of Hevea (to take the most obvious example) is very irregular or may be absent, and there is no such general flowering of trees and shrubs as in dry years. One result is that the fruit crop may fail almost completely. In some years there may be a somewhat dry period about June–July, which may cause a less obvious development of dry-season phenomena. The giant orchid, *Grammatophyllum speciosum*, normally flowers in Penang in July and August. In my experience it is much less regular in Singapore, omitting its flowering, or flowering much later, in those years when there is a marked dry season early in the year. There may be all gradations between a dry season (three weeks without rain have been recorded on a few occasions) and a very wet one; in the moderately rainy cases there is a great irregularity of behaviour among plants of the same species, seen especially in Hevea on account of its abundance.

In view of the above observations, I think that there is no doubt that many plants alter their behaviour from year to year in Singapore according to the climatic conditions; though of course this may only be exhibited by a conspicuous minority of species. In his discussion referred to above, Coster concludes, with Klebs, that there is no definite inner rhythm of the plant, but that periodic phenomena are always due to the interaction of external conditions with the inner condition of the living tissues of the plant. It certainly appears that the behaviour of many plants in Singapore supports this view, and that the very irregular or almost non-seasonal climate offers a most promising field for the observation of these phenomena in relation to climatic changes. A comparison of such observations with those from more definitely seasonal localities would undoubtedly be of great interest.

May 16th, 1927

SOME STRUCTURAL CHARACTERS OF THE GENUS *DICTYONEMA* HALL, AND THE TECHNIQUE EMPLOYED IN THEIR DETERMINATION

By O. M. B. Bulman

In this paper, the opportunity was taken of referring, in advance of detailed publication, to some recent work on the structure of the dendroid graptolite *Dictyonema flabelliforme* (Eichwald) and of examining these observations in the light of previous work on the genus. A brief review was first given of the methods of technique employed in the determination of the structure of these organisms.

From sections of well-preserved material of the Cambrian species, *Dictyonema flabelliforme*, the stipe is now known to be composed of the three types of individuals recognised by Wiman in the Silurian species *D. rarum*, *D. peltatum* and *D. cavernosum*, since there is definitely something corresponding to the budding-individual of these later forms.

Wiman showed (*Bull. Geol. Inst. Upsala*, III, 1896, p. 1) that the rhabdosome of *D. cavernosum* was produced from a stem with well-developed root-like processes. On sectioning the proximal portion, he discovered that the colony commenced with two individuals, one of which was a hydrotheca and the other a budding individual. He put forward a number of hypotheses to explain this association of two individuals, and one of the most favoured was that the hydrotheca was older and had produced the budding individual. He concluded that, in this event, the hydrotheca was not a true hydrotheca, but was morphologically comparable with the sicula of a graptolite. Information obtained by sectioning the proximal portion of *D. flabelliforme* supports this explanation, since it is shown that the lateral bud given off at an early stage from the virgella side of the sicula is functionally a budding individual. The conclusion is drawn, from this and other facts, that the two adhesive structures—stem and nema—are not fundamentally distinct, but rather that one is a modification of the other.

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SOME PROBLEMS IN THE COMPARISON OF CHROMOSOMES

By J. S. YEATES

THIS paper was a preliminary account of some work which has been carried out with a view to developing technique for chromosome comparison. In plants, chromosome phylogenies based on numbers of chromosomes almost invariably run from low numbers to high numbers in each genus (*Rosa*, 14, 28, 35, 42, 56; *Carex*, 9, 15, 16, 19...56). Some other comparative basis is necessary by which we can take into account the possible fusion of chromosomes or the gradual diminution of their material. Some such process must occur, for in spite of the frequency of genera with ascending series, most genera appear to include some species which have low numbers.

The external morphology of chromosomes is of limited use in comparison, the occasional presence of satellites being the most useful feature. Delaunay(1) has shown how a series of species in *Muscari* can be arranged so as to show progressive disappearance of satellites—a feature which he correlates with loss of fertility in the plant.

The writer has attempted to use the internal structure of chromosomes for comparison. Baranatsky(2), Kaufmann(3), and Kuwada and Sugimoto(4) have described a spiral structure in chromosomes of *Tradescantia*. Using Taylor's fixed smear method, the writer has observed a similar structure in reducing pollen mother cells of *Tradescantia virginica*, *Hyacinthus orientalis*, *Kniphofia Nelsoni*, and *Bomarea cantabrigiensis*. A spiral structure was found also in anaphase chromosomes of the root tip of *Vicia Faba*. Kuwada(5) has also recorded it for this plant. Up to the present time this spiral structure has not been found of use in comparison.

Measurements of chromosome size gave more promising results. As an object for study, the short segment on one limb of the *m*-chromosome of *Vicia Faba* was chosen. Seeds of this plant were grown in sand at 23° C., the roots fixed and stained by the same methods, and the chromosome segments drawn and measured at a magnification of 2850. The following were the chief conclusions: (*a*) in a single plant the length of the segment varied from 6·5–10 units; (*b*) the mean length in different roots of the same plant varied by less than 1 per cent.; (*c*) in a single population (garden variety) the mean length in different plants fell sharply into two groups; (*d*) in the species *V. Faba*, seven varieties have so far been examined; the mean length of the segment varies from 7·7 $\mp$ 0·05 units for one variety to 10·8 $\mp$ 0·045 units for another.

In view of these results, it is clear that the mean value for the length of a certain chromosome may vary considerably within a single species. Extreme caution is therefore necessary in making comparisons of size between separate species. It appears also that while certain mean lengths recur in some (apparently impure) samples, different varieties give generally different mean lengths. It remains to be decided whether or not mean chromosome length is inherited, and if it has the same value for all the individuals of a pure line.

REFERENCES.

(1) DELAUNAY, L. (1915). *N. Pr. Mem. Soc. Natur. Kiew*, **25**.
(2) BARANATSKY, J. (1880). *Bot. Zeit.* **38**.
(3) KAUFMANN, B. P. (1926). *Amer. Journ. Bot.* **13**.
(4) KUWADA, Y. and SUGIMOTO, T. (1926). *Bot. Mag. Tokyo*, **40**.
(5) KUWADA, Y. (1926). *Mem. Coll. Sci., Kyoto Imp. Univ.*, Ser. B, **2**.